THE EFFECT OF DIVISION OF THE ANTERIOR AND POSTERIOR ROOTS OF THE SPINAL CORD ON THE MITOTIC ACTIVITY OF THE EPIDERMIS AND THE RATE OF HEALING OF CUTANEOUS WOUNDS IN RATS

A. N. Kulagin

Laboratory of Histophysiology (Head—Candidate Biol. Sci. V. N. Dobrokhotov)
of the Institute of Experimental Biology (Director—Prof. I. N. Maiskii) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
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We have previously shown [5, 6] that unilateral division of the nerves of the hindlimb (femoral, obturator and sciatic) leads to a decrease in the mitotic activity of the epidermal cells and to a slowing of the healing of cutaneous wounds in the denervated limb.

Since the nerves divided were mixed, it was impossible to discover on account of which fibers—afferent or efferent— it was that their exclusion led to these changes.

The existing data [1, 3, 9] is limited and contradictory, and does not permit any definite conclusions to be drawn about the influence of the afferent and efferent innervation on the mitotic activity of the tissues and the rate of wound healing. Meanwhile, the investigation of these problems is of great importance to the elucidation of the laws governing the regulation of regenerative processes and cell division.

In the present work we studied the effect of either purely afferent or purely efferent nerves on cellular proliferation and on the rate of healing of skin wound.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male white rats (average weight 150 g). Depending on the character of the operation, the rats were divided into three groups, each of seven animals. In the animals of the first group, the anterior roots of the spinal cord were divided on the left side, the posterior roots being preserved; in animals of the second group both anterior roots were divided; finally, in the animals of the third group, the posterior roots were divided and the anterior roots left intact.

Division of the roots was carried out under ether anesthesia, by the method suggested by G. N. Kryzhanoskii. In all cases the last thoracic, all the lumbar and the first sacral roots were divided. In the postoperative period tests of the completeness of the denervation were performed on two occasions by means of the application of painful stimuli to the denervated and intact limbs.

On the tenth day after operation a piece of skin for counting the number of mitoses was excised from the lateral aspect of the upper third of both legs in all the animals. The pieces of skin measured 4 · 8 mm. The wounds thus formed were used for the subsequent study of the rate of wound healing. Simultaneously with the infliction of skin wounds to the hindlimbs of animals on which the operation of division of the anterior or posterior roots had been carried out, wounds of the same size were also inflicted to the forelimbs in the region of the forearm. The material for counting mitoses was fixed in Bouin's fluid and embedded in celloidin-paraffin wax. Sections 7μ in thickness were stained with hematoxylin by Carazzi's method. The mitotic activity and the rate of healing of cutaneous wounds were estimated by the methods described in our previous papers [4, 5]. The observations were continued for a period of 40 days after denervation.

TABLE 1. Mitotic Coefficient in the Epidermal Cells of the Skin of Rats after Division of the Anterior Roots of the Spinal Cord

Mitotic coefficient (in ‰)		Difference between mitotic coefficients of limbs as a percentage of intact limb
in the intact limb	in the denervated limb	
10,3	7,3	70,87
9,9	5,4	54,54
7,9	2,3	29,12
7,3	6,2	84,93
6,3	3,8	60,31
5,8	3,8	65,51
5,2	5,6	107,69
Mean 7,6	4,9	64,5

TABLE 2. Mitotic Coefficient in the Epidermal Cells of the Skin in Rats after Division of the Anterior and Posterior Roots of the Spinal Cord

Mitotic coefficient (in ‰)		Difference between mitotic coefficients of limbs as a percentage of intact limb
in the intact limb	in the denervated limb	
6,5	4,7	72,30
6,4	1,6	25,00
5,8	1,7	30,00
5,7	2,6	45,61
5,1	2,5	49,01
5,0	4,2	84,00
3,0	1,9	63,33
Mean 5,4	2,7	50,00

EXPERIMENTAL RESULTS

Figures showing the mitotic activity in rats after division of the anterior roots of the spinal cord are given in Table 1, from which it is clear that the mitotic coefficient in the epidermis of the denervated limbs is lower in nearly every case than in the epidermis of the intact limbs.

The difference between the mean mitotic coefficients was significant (P= 0.01). Identical results were obtained after the simultaneous division of both anterior and posterior roots (Table 2). In this case too, the difference between the mean values was significant (P= 0.001).

Counts of the number of mitoses after division of the posterior roots showed that the mean mitotic coefficient in the two limbs was almost the same: In the limbs with afferent nerves intact it was 4.1 ‰, and in the limbs with afferent nerves destroyed it was 4.2‰ (Table 3).

It must be pointed out, however, that in the rats in which the anterior roots were divided , the mitotic activity in the epidermis of both limbs was considerably higher than in the animals in which both anterior and posterior roots had been simultaneously divided. This difference is close to significant: Its value in the denervated limbs was P = 0.02, and in the intact limbs P = 0.03.

The number of the different phases of mitosis in the epidermis of the limbs with disturbed innervation showed no essential difference from their number in the epidermis of the intact limbs. This showed that the division of the anterior or posterior roots of the spinal cord, just as the simultaneous division of both, does not alter the rate of the course of any of the phases of mitosis. It may thus be considered that the decrease which was found in the total number of mitoses in the epidermis of the denervated limbs was dependent on the smaller number of cells taking part in division and not on any increase in the duration of the process of cell division itself, at any of its phases.

The study of the process of healing of cutaneous wounds in the rats with division of the anterior roots of the spinal cord showed that the wounds of both fore- and hindlimbs healed in roughly the same periods of time (17-20 days).

In rats in which the anterior and posterior roots had been simultaneously divided, wounds were inflicted on the hindlimbs alone. In this group of animals, healing of the wounds of the intact limbs took place without any apparent abnormality and was complete on the 10th-12th day. Soon after the infliction of wounds on the thigh and leg of the denervated limbs in two rats, ulcers developed, which showed rapid progression. Gangrene of the limb ultimately developed, and this was probably the cause of their comparatively rapid death (on the 6th-8th day). In one rat a large ulcer formed at the site of the wound, and in two other rats the wounds neither healed nor increased in size. In the remaining two animals the wounds healed on the 18th-20th day.

The simultaneous division of the anterior and posterior roots thus greatly complicated the process of wound healing or led to the formation of trophic ulcers.

In animals in which the posterior roots of the spinal cord had been divided, the wounds on the forelimbs and on the hindlimbs with their intact afferent nerve supply healed at approximately the same rate (10-12 days).

TABLE 3. Mitotic Coefficient in the Epidermal Cells of the Skin in Rats after Division of the Posterior Roots of the Spinal Cord

Mitotic coefficient (in ‰)	
in the intact limb	in the denervated limb
8,1	2,2
7,3	10,0
6,1	5,1
2,8	3,1
1,9	6,6
1,6	0,9
1,2	0,3
Mean 4,1	4,2

On the limbs deprived of their afferent nerve supply, in all the rats except one, ulcers developed at the site of the wounds. It must be pointed out that, in these same rats, ulcers were also formed at the back of the foot of the limbs on the site of the posterior root section, at different intervals of time after the operation (on the 19th-32nd day), in those areas which were most exposed to trauma during the animal's movements.

Under experimental conditions, the formation of trophic ulcers on the foot was thus observed only in those animals in which the posterior roots had been divided, which demonstrates the important role of the afferent innervation in trophic disorders of the tissues. Our findings were in agreement with the views of T. A. Grigor'eva [2], who considers that trophic disorders arise in the tissues as the result of the disturbance of the function of the peripheral afferent neurone, and that these processes are directly dependent on the degree of destruction of the afferent nerve supply. I. D. Khlopina [7, 8] maintains another point of view namely that the disturbance of the trophism of the tissues does not depend on the exclusion of a particular component of the nervous system, but on the degree of denervation. According to her findings, the development of trophic ulcers is observed only in those cases when the roots are divided distally to the spinal ganglion.

In our experiments, the posterior roots were divided proximally to the ganglia, yet nevertheless ulcers were formed in six of the seven rats. The principal condition which could lead to the development of ulcers of the foot , in our opinion, was the additional irritation which resulted from the excision of pieces of skin for counting of the mitoses. This hypothesis is in full agreement with the views of A. D. Speranskii [6] concerning the "second blow".

The development of trophic ulcers in animals after section of the posterior roots and after the simultaneous section of the anterior and posterior roots, and the absence of ulcers after section of the anterior roots alone, is evidence that this process is not dependent on the degree of denervation, but on which roots are divided.

SUMMARY

Unilateral section of the anterior roots of the spinal cord in rats leads to the reduction of mitotic activity of the epidermal cells in the limb thus deprived of its afferent nerve supply and has no significant effect on the rate of wound healing. Section of posterior roots does not produce any change in the number of dividing cells in the epidermis of the extremities but results in the development of trophic ulcers. Simultaneous division of both anterior and posterior roots of the spinal cord leads to reduction of mitotic activity of the epidermal cells in the operated extremity; in a number of cases it provokes the formation of trophic ulcers at the site of the wound infliction or the development of gangrene.

Our results thus demonstrate that the exclusion of a particular form of innervation differs in its effect on the mitotic activity of the epidermal cells and on the rate of healing of cutaneous wounds.

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