A PAIN SYNDROME OF SPINAL ORIGIN

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A pain syndrome was reproduced in experiments on albino rats by injecting purified tetanus toxin into the region of the posterior horns of the lumbosacral segments of the spinal cord. During development of the syndrome an attack of pain was provoked by applying stimuli to the corresponding projection zone of the hindlimb or tail, and it was accompanied by a cry, by licking, and later by biting the tissue of that zone, by changes in respiration, elevation of the blood pressure, and dilatation of the pupil. At the height of development of the syndrome attacks occurred without special provocation. It is concluded that the syndrome is based on the formation of a generator of excitation, as a result of disturbance of the inhibitory mechanisms, in the segmental system of ascending tracts of pain sensation, resembling the "departure station" phenomenon described previously.

Previous investigations [2-5,7, 8] have shown that a powerful generator of excitation can be formed in the spinal cord with the aid of tetanus toxin, which disturbs various types of inhibition [2,6,9,11,13]. This generator of excitation consists of a group of interneurons with disturbed inhibitory mechanisms. Such a generator is triggered by impulses traveling along certain afferent channels and it plays the role of a "departure station"; excitation sent by it, because of its intensity, reaches different parts of the CNS; the system in which the departure station is created is thereby activated (the "departure station" phenomenon) [2-4].

The object of the investigation described below was to study the possibility of forming a departure station in a system related to the tracts of pain sensation in the spinal cord, and thus to reproduce a pain syndrome of spinal origin.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 270-300 g. To ensure precision of injection of the toxin a stereotaxic apparatus with microinjector (designed by G. N. Kryzhanovskii and A. I. Bartyzel') was used; with the spin fixed rigidly above and below the site of exposure of the spinal cord (with the animal's abdomen hanging down), fluid could be injected in a volume of 10^{-4} ml. The microinjector consists of a syringe the plunger of which is connected to a micrometer. A glass micropipet with a tip having an external diameter of 20-30 μ is fixed over the needle of the syringe; the pipet is fixed to the needle with Mendeleev's paste.

Experiments were carried out under ether anesthesia. The spinal cord was exposed in the region of the second and third lumbar vertebrae, where the lumbosacral enlargment is located. To avoid unnecessary trauma the spinal cord was exposed only on the side of injection of the toxin, leaving the overlying bone intact on the opposite side. Toxin was injected into the region of the posterior horn, at the origin of the dorsal roots, by 1, 2, or 3 punctures. The depth of insertion of the micropipet was estimated from micrometer readings, having first been determined in special experiments with injection of dye; in the volume

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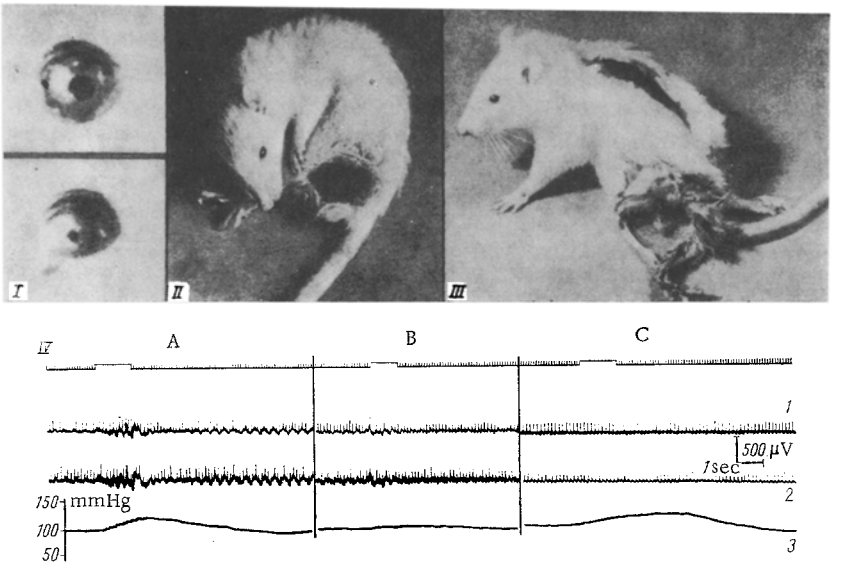

Fig. 1. Appearance of a pain syndrome evoked by injection of tetanus toxin into posterior horns of lumbosacral segments: I) reaction of pupil to tactile stimulation in area of left hindlimb (on side of injection of toxin) which the animal licks, and to application of similar stimulus to symmetrical area of opposite limb; II) animal's response to touching area which it licks vigorously; as a result of licking the hair is removed from that part, the skin ulcerated and the dermis exposed; III) one of the final stages of the pain syndrome: the soft tissues of the limb are chewed on the side of injection of the toxin and the bone exposed; IV) response of respiration and blood pressure to application of tactile stimulus to area licked (A), to application of the same stimulus to symmetrical area of opposite limb (B), and to application of stimulus to area licked after complete curarization of the animal and artificial respiration (C). Curves from top to bottom: time marker and marker of stimulation (together); electrical activity of intercostal muscles on left (1) and right (2), blood pressure (3).

chosen (10^{-4} ml) the dye was localized in the region of the posterior horn, most of it lying in the region of Rexed's laminae IV-V (results of investigations on rats [24]).

The toxin was injected in doses of 0.1-3 MLD (for rats of that weight). Liquid, purified, concentrated toxin was used, and was made up from dry, unpurified toxin by gel-filtration in 0.1 M phosphate buffer, pH 7.0, on a column with Sephadex-G-100. The specific activity of the purified toxin was $(1.8\text{-}2) \cdot 10^{-5}$ MLD mg protein. More than 150 animals were used in the experiments.

EXPERIMENTAL RESULTS

Injection of tetanus toxin into the region of the posterior horns of the lumbosacral segments of the spinal cord led to the appearance of a series of characteristic symptoms and phenomena which together can be regarded as a pain syndrome. Its development was characterized by the following features.

A certain interval of time after injection of the toxin (the length of this interval depended on the dose of toxin) the animals became excited and aggressive and they attacked each other. They then began to lick their hair on an area of the hindlimb on the side of injection of the toxin. Application of comparatively weak nociceptive stimuli to that area evoked a considerable response: the animal cried and began to lick the part vigorously. Similar stimuli applied to the symmetrical area of the opposite limb did not evoke the same response. With the course of time the rats licked and bit the skin of that same area more and more frequently and vigorously, so that some of the hair was removed, the skin became ulcerated, and the dermis was exposed (Fig. 1, II). During this period the increased sensitivity in that affected area became particularly marked; the slightest stimulus, sometimes even a gentle tactile stimulus (touching with a bristle, stroking it over the hair, or blowing on the limb) induced a marked response: the animal turned sharply on the limb with a groan and a cry, bit it furiously, and even started to chew the tissues. The response was character-

ized by a considerable after-effect: it continued for a long time after the application of the stimulus. During this period unconnected stimuli from other parts of the body, tapping, beating on the table, and so on, evoking a general response of the animal, could also provoke the attack described. With the course of time the area of increased sensitivity grew larger, the animal's response became more and more furious, and the attacks of pain occurred increasingly often and apparently spontaneously. If the process continued, sometimes the animal chewed the tissues of its own limb (Fig. 1, III).

The area of increased sensitivity from which this response was provoked and which it first licked vigorously and then sometimes chewed also, was most frequently situated on the lateral surface of the thigh or leg. However, it could be found on either the posterior or anterior surface of the thigh or leg and on the foot. Its localization depended on the way the toxin was injected. A series of experiments showed that this area could also arise on the tail if the toxin was injected into the posterior horn of the sacral segments.

The behavioral response as described above was accompanied by a group of autonomic symptoms: in response to gentle tactile stimulation of the area of hyperalgesia specified, dilatation of the pupil, protrusion of the eyeball, widening of the palpebral fissure (Fig. 1, I), holding the breath and disturbance of respiration, and elevation of the blood pressure unconnected with the motor response were observed (Fig. 1, IV).

Postmortem examination revealed macroscopic changes in the internal organs: severe paralytic dilatation of the heart, hyperemia of the kidneys and liver and, in particular, of the lungs. Histological examination of the internal organs showed considerable leukostasis.

Special investigations [1] showed that during development of this syndrome there was an initial, transient increase followed by a decrease in the catecholamine concentration in the internal organs, especially the heart and adrenals.

It can be concluded from this description that injection of tetanus toxin into the region of the posterior horns of the spinal cord evokes a typical pain syndrome.

To understand the nature of this syndrome and to study it as a model with which to investigate the spinal mechanisms of pain, it was particularly important to determine whether its origin is connected with the specific action of the tetanus toxin or whether it is due to injury to the spinal cord. This problem became particularly acute after the first investigations of tetanus [18, 23], which showed that after injection of tetanus toxin into the region of the posterior horns of the spinal cord the condition known as painful tetanus may develop. This type of pathology has been reproduced by several workers [16-19], but with difficulty and very rarely, and the view has been expressed [26] that it is a technical artefact and the result of injury to the spinal cord or dorsal roots. To examine this problem a series of special investigations was carried out; they showed that injection of inactivated tetanus toxin in the same way into the spinal cord did not cause the appearance of a pain syndrome. The production of this pain syndrome described above was thus connected with the specific action of the tetanus toxin.

The pain syndrome under examination is spinal in origin; it is caused by functional changes in the segmental apparatus and not in the supraspinal levels. This is shown by experiments with transection of the spinal cord: after transection (at the level of segments T4-5) the pain syndrome disappears and the segmental component of the process is clearly manifested as an increase of flexor tone. Later, in connection with the arrival of the toxin in the region of the anterior horns [2], the characteristic extensor rigidity of tetanus develops.

What are the mechanisms of the pain syndrome described above? Since tetanus toxin disturbs various types of inhibition in the spinal cord and has no direct excitatory influence on neurons [2, 9, 11], it can be postulated that this syndrome is based on a disturbance of the inhibitory mechanisms regulating the intensity of the sensory flow, relaying to the ascending tracts of pain sensation. Such inhibitory mechanisms have now been described [10, 12, 14, 15, 20-22, 25]. However, this is not a question purely of facilitation of conduction through a sensory relay, as might follow from the concept of "gate control" [21, 22]. The distinguishing features of the phenomenon described above suggest that disturbance of the inhibitory mechanisms creates a powerful generator of excitation in the system of the sensory relay, giving rise to a powerful and prolonged flow of impulses along the ascending tracts. The morpho-functional characteristics of the posterior horn and, in particular, of lamina V, in which neurons responsible for transmission, convergence, summation and augmentation of excitation and inhibition are concentrated [20, 22, 25], and also the presence

of multiple connections between neurons located in the posterior horn confirm the view that such a generator can actually arise. This generator is a group of interconnected neurons with disturbed inhibitory mechanisms. The more disturbed the inhibitory mechanisms and the more numerous the interneurons generating the excitation which are involved in the process, the greater the power of such a generator. For this reason the pain syndrome described above increases progressively in its intensity as the toxin spreads over the structures of the posterior horn. For the same reason the area of skin from which the attack of pain can be provoked becomes wider and the thresholds of its production are lowered. The group of interneurons with disturbed inhibitory mechanisms forming the generator is not a focus of constant excitation; this system leads to a state of preparedness for an explosive burst of excitation which arises in response to the arrival of impulses along the appropriate afferent channel. These impulses play the role of triggering stimulus for the generator: once excited it works independently and requires no reinforcing stimulation from the periphery. It is a striking fact that the attack of pain can be provoked from the appropriate area of skin even by gentle tactile stimuli. This is evidence that the generator can be triggered by stimuli of different modalities. At the height of development of the syndrome the generator may apparently work in accordance with the "all or nothing" rule. In the same period the generator can be triggered by convergence of impulses from other sources; the thresholds of its excitation are sharply reduced, so that an attack can arise even though not specially provoked.

The pain syndrome described above is thus an example of the "departure station" phenomenon [2-5]. This feature is manifested very clearly in particular in the early stages of its development. Besides its neurological importance as a pain syndrome of spinal origin, this syndrome can also be used as an interesting model with which to study the mechanisms of pain.

LITERATURE CITED

1. G. N. Kassil', G. N. Kryzhanovskii, É. Sh. Matlina, et al., Dokl. Akad. Nauk SSSR, 204, No. 1, 246 (1952).
2. G. N. Kryzhanovskii, Tetanus [in Russian], Moscow (1966).
3. G. N. Kryzhanovskii, in: The Integrative Activity of the Nervous System under Normal and Pathological Conditions [in Russian], Moscow (1968), p. 21.
4. G. N. Kryzhanovskii, Pat. Fiziol., No. 3 (1973).
5. G. N. Kryzhanovskii and V. K. Lutsenko, Byull. Éksperim. Biol. i Med., No. 8, 25 (1970).
6. G. N. Kryzhanovskii and F. D. Sheikhon, Byull. Éksperim. Biol. i Med., No. 11, 9 (1968).
7. V. K. Lutsenko and G. N. Kryzhanovskii, Byull. Éksperim. Biol. i Med., No. 6, 6 (1973).
8. V. K. Lutsenko and G. N. Kryzhanovskii, Byull. Éksperim. Biol. i Med., No. 7, 21 (1973).
9. Yu. S. Sverdlov, Neirofiziologiya, 1, No. 1, 25 (1969).
10. D. Albe-Fessard, J. Physiol. (Paris), 60, Suppl. 1, 1 (1968).
11. V. Brooks, D. Curtis, and J. Eccles, J. Physiol. (London), 135, 655 (1957).
12. D. Carpenter, J. Engberg, and A. Lundberg, Acta Physiol. Scand., 63, 103 (1965).
13. D. Curtis et al., Brain Res., 10, 208 (1968).
14. B. N. Christensen and E. N. Perl, J. Neurophysiol., 33, 293 (1970).
15. J. Engberg, A. Lundberg, and R. W. Ryall, J. Physiol. (London), 187, 418 (1966).
16. W. Firor and A. Lamant, Ann. Surg., 108, 941 (1938).
17. W. Firor, A. Lamant, and H. Shumacker, Ann. Surg., 111, 246 (1940).
18. W. Fletcher, Brain, p. 383 (1903).
19. A. Fröhlich and H. Meyer, Arch. exp. Path. Pharmak., 79, 55 (1916).
20. P. Hillman and P. D. Wall, Exp. Brain Res., 9, 284 (1969).
21. R. Melzack and P. Wall, Science, 150, 971 (1965).
22. R. Melzack and P. Wall, Int. Anesthesiol. Clin., 8, 3 (1970).
23. H. Meyer and F. Ransom, Arch. exp. Path. Pharmak., 49, 369 (1903).
24. T. Y. Stener and L. M. Turner, J. Physiol. (London), 222, No. 2, 123P (1972).
25. P. D. Wall, J. Physiol. (London), 188, 403 (1967).
26. A. Zirani, Boll. Ist. Sieroter. Milan, 30, 435 (1951).