Analgesic Effects of Spinal Cord Peptide Factors Produced During the Establishment and Development of Generators of Pathologically Enhanced Excitation in the Rat Spinal Cord

G. N. Kryzhanovskii, E. I. Danilova, V. N. Grafova, and M. Yu. Karganov

UDC 616.832-092.9-02:615.212]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 10, pp. 374-377, October, 1994
Original article submitted January 28, 1994

In rats with a pain syndrome caused by the production of a generator of pathologically enhanced excitation, substances of peptide nature with analgesic properties were found to be produced in the nociceptive system of the spinal cord. Spinal cord extracts derived from rats with such syndromes (pain syndrome of spinal origin or adjuvant arthritis) exerted analgesic effects when injected intraventricularly into recipient rats with a pain syndrome of spinal origin. The highest analgesic activity was displayed by extracts obtained from the region where the generator of pathologically enhanced excitation had been set up. The analgesic activity of the extracts was directly related to the severity and duration of the pain syndrome in the donor rats.

Key Words: *spinal cord peptides; analgesics; pain syndrome; adjuvant arthritis*

When a disease process arises in the nervous system, substances that either promote or inhibit this process are elaborated by populations of the involved hyperactive neurons, which form aggregates acting as a generator of pathologically enhanced excitation (GPEE) [2]. For example, pro- or antiepileptic substances of peptide nature were identified in the brain of rats during the intensification of convulsive activity in the event of kindling [3,6]. Presumably, the emergence of substances of a particular type reflects activation either of the pathological system underlying the disease process or of an antisystem that limits its development. Thus, when cerebellar nuclei pertaining to an antiepileptic system were activated, epileptic activity was suppressed or weakened, and substances capable of depressing epileptic activity in recipient animals were then detected in the brain and cerebrospinal fluid of donor animals [7]. Similar substances have been found to appear in animal and human cerebrospinal fluid after an epileptic seizure [3,7]. The present study was designed to determine which substances - pro- or antiepileptic - occur in the spinal cord when a pain syndrome (PS) of spinal origin is developing in rats under the influence of the GPEE formed in the dorsal horns of lumbosacral segments.

MATERIALS AND METHODS

A total of 422 random-bred white rats weighing 200-220 g were used. An acute PS was induced in test rats by creating a GPEE in spinal cord horns

Laboratory for General Pathology of the Nervous System and Laboratory for Pathophysiology of Pain, Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

0007-4888/94/0010-1075$12.50 ©1995 Plenum Publishing Corporation

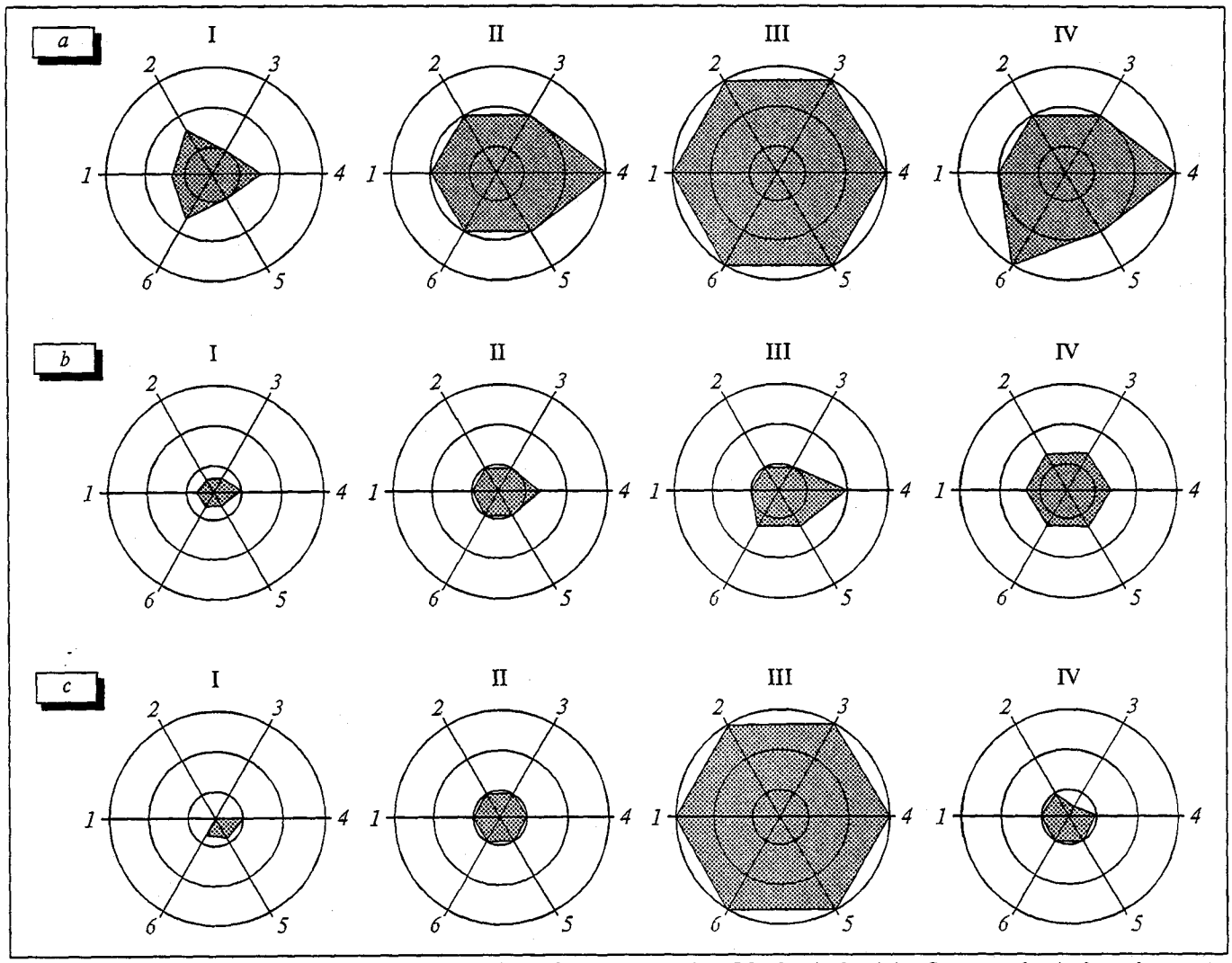

Fig. 1. Analgesic activity of spinal cord extracts derived from rats with a PS of spinal origin. Sources of spinal cord extracts: *I*) lumbar segments of both cord halves; *II*) "generator" half of lumbar segments; *III*) contralateral half of lumbar segments; *IV*) thoracic segments of both halves. PS duration after tetanus application: *a*) 3 h; *b*) 5–6 h; *c*) 15 h. Here and in Fig. 2 characteristics of the PS are depicted by radius vectors as follows: *1*) number of attacks of pain per minute; *2*) duration of one attack (sec); *3*) intervals between attacks (sec); *4*) response to provocation (scores); *5*) vocalization (score 1 assigned to a weak, short squeak and score 3, to sharp, shrill squeaking throughout the attack); 6) motor activity (score 1 assigned to one or two runs during an attack of pain and score 3, to running about the cage with leaps throughout the attack). Degree of the PS, i.e., the magnitude of its manifestations, was scored as follows: 0, none; 1, slight; 2, moderate; 3, severe. In these diagrams each circle corresponds to one score, counting from the center.

on the left at the L_4-L_6 level with benzylpenicillin sodium or tetanus toxin. For this, benzylpenicillin sodium or tetanus toxin were embedded in liquid agar and, after its solidification, a 1×0.4×10 mm plate containing 8000 IU of benzylpenicillin sodium or 8-10 LD_{min} (minimal lethal doses) of tetanus toxin was prepared and applied to the dorsal surface of the left half of the spinal cord following a unilateral laminectomy at the L_4-L_6 level under ether anesthesia. The rats developed a PS 10-15 min after benzylpenicillin sodium application (i.e., immediately after recovering from the anesthesia) and 2-3 h after tetanus toxin application. The severity of the PS, which lasted 3 h and 15-18 h after benzylpenicillin sodium and tetanus toxin, respectively, was assigned scores 1 to 3. Rats with the PS caused by tetanus toxin were used as donors of spinal cord extracts, while those with the PS induced by benzylpenicillin sodium were used as recipients of these extracts for a study of their analgesic effects. The donor rats were decapitated 3, 5, and 15 h after tetanus toxin application and their spinal cord was extirpated both at the application site and in the area of remote thoracic segments. Cord tissue samples were frozen and stored at -20°C until use. For peptide extraction, frozen cord tissue samples were placed in a glass homogenizer containing 1 M acetic acid

heated to 90°C, and the homogenizer was kept in a boiling bath for 5 min, after which the tissue was dispersed with a glass pestle during 10 min [8,9]. The suspension was cooled, allowed to stand for 30 min, and then centrifuged at 10,000 *g* for 15 min. The supernatant was collected and its pH was adjusted to 7.0 with concentrated ammonia solution. The sediment was separated by repeated centrifugation and the resulting solution was lyophilized. Extracts obtained from both halves of the spinal cord (lumbar and thoracic regions) and from one of its halves - the side where the GPEE had formed ("generator" side) or the contralateral side - were assayed for analgesic activity. Sham-operated rats with a plate made of 1% agar applied to the spinal cord served as controls. For some tests, spinal cord extracts were treated with Pronase (Serva) to confirm the peptide nature of the active principle in the extracts [4,5,10].

In other test rats, a PS was produced by Freund's adjuvant injected in a dose of 0.1 ml into the sole of the left foot (these rats developed adjuvant arthritis), and its severity was assessed by measuring the degree of edema in the limb and by observing the spread of edema to other limbs.

Analgesic activity of the extracts was estimated after an intraventricular injection (into the fourth ventricle) of their lyophilizates in solution (5 μg protein in 10 μg solution per animal) in rats with the PS caused by penicillin application to the spinal cord as described above. The results were treated statistically using Student's *t* test.

RESULTS

The PS arising in rats 10-15 min after penicillin application to the spinal cord was manifested by characteristic behavioral acts, including licking and biting of the pain projection area (left hind limb), vocalization, enhanced motor activity, anxiety, and excitation. Spontaneous (unprovoked) spells of pain occurred at a frequency of 3 or 4 per minute, lasted 10-13 sec each, and were accompanied by squeaking and fierce assaults upon the left hind limb (pain projection area). Occasionally, the attacks of pain were so frequent that the intervals between them were difficult to record. Such attacks were assigned score 3.

The spinal cord extracts derived from rats with the PS produced analgesic effects in the recipients with the PS of spinal origin: the attacks of pain were less frequent and much less intensive, and the animals became calm, squeaked only rarely and for short periods, and just touched the hind limb without biting it. The analgesic activity of

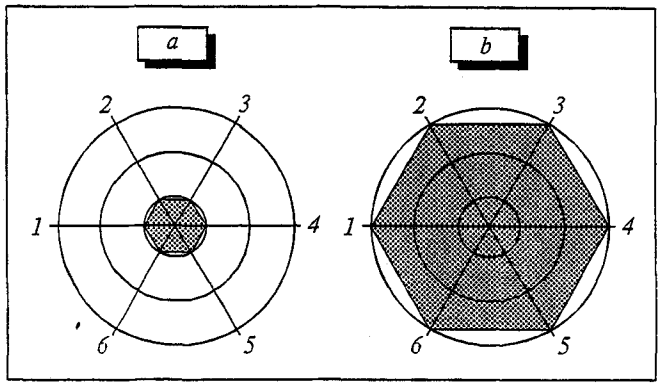

Fig. 2. Analgesic activity of spinal cord extracts derived from rats with a PS related to adjuvant arthritis. *a*) extract from the left half of lumbar segments (L_{5-6}); *b*) extract from the right half of lumbar segments. Freund's adjuvant was injected into the plantar surface of the left hind limb.

lumbar extracts from both spinal cord halves was the higher, the more severe and longer-lasting the PS in the donor rats (Fig. 1, *I*, *a*, *b*, and *c*). The analgesic activity of extracts from the thoracic cord region (far away from the GPEE in the lumbar segments) was weaker but also increased with increasing duration of the PS in the donor rats (Fig. 1, *IV*, *a*, *b*, and *c*).

The analgesic activity of cord extracts from the side of convulsant application (i.e., the site where the GPEE had formed) and from the contralateral side of rats with a PS lasting for 5-6 h was similar (Fig. 1, *II*, *d* and *III*, *b*). The analgesic activity of cord extracts from the side of GPEE formation in rats with a longer-lasting PS was roughly the same as above (Fig. 1, *II*, *c*), while that of cord extracts from the contralateral side of such rats was much lower or zero (Fig. 1, *III*, *c*).

Cord extracts derived from control rats in which an agar plate without convulsant was applied to the dorsal cord surface failed to exhibit analgesic activity or to suppress the PS in the recipients. Pronase-treated extracts were also devoid of analgesic activity.

Rats injected with Freund's adjuvant into the left hind paw developed a PS in 2 to 3 weeks. The paw became swollen and ulcerated. The animals favored the affected paw in walking (they held it in the air), preferred sitting at a distance from each other, and displayed aggressivity if they happened to touch each other while moving around. They responded to mechanical stimulation of the affected paw by jerking it away, squeaking, and licking the swollen area. The extract derived from the lumbar region on the side of adjuvant injection showed analgesic activity after its injection into rats with the PS of spinal origin (Fig. 2, *a*), as was evidenced both by much less frequent

episodes of spontaneous pain and by very short durations (2-3 sec) of provoked pain, which was accompanied by a single squeak and a run over a short distance. The extract from the contralateral side lacked analgesic activity (Fig. 2, *b*).

As a result of neuronal hyperactivation in the nociceptive system of the spinal cord, substances that mediate functional effects of this system accumulate in the GPEE area and exert an analgesic effect upon injection into recipient animals. Tests with Pronase showed these substances to be peptides. The failure to display analgesic activity by extracts derived from the contralateral spinal cord side of rats in which the PS had lasted for only a short time (3 h) and the weak analgesic activity of extracts derived from cord regions remote from the GPEE in such rats indicate that active factors were primarily accumulating in the GPEE area during the first few hours of PS development. However, extracts from the contralateral side did possess some inherent activity given that the analgesic effects of extracts prepared from both halves of the spinal cord were greater than those of extracts prepared from its "generator" half only. Moreover, marked analgesic activity was exhibited not only by extracts from the "generator" side of the spinal cord but also by those from the contralateral side, as well as by those from the thoracic segments if the material used to prepare the extracts was taken in a later phase (at 5 h) of PS development.

Measurements of enkephalins in various spinal cord regions of rats with a PS demonstrated considerable elevations (by 150%) of their levels in the area of GPEE set up in lumbar segments, less marked elevations (by 60%) in thoracic cord segments, and still less marked (by 35%) in the contralateral half of the spinal cord [1]. When a GPEE is set up in the nociceptive system of lumbar segments, this region becomes hyperactive and begins functioning as a pathological determinant [2]. Other parts of the central nervous system may become pathological determinants themselves after they have been subjected to the original determinant for a sufficiently long time. Such a situation was observed in the present study: extracts prepared from thoracic cord segments of rats with a PS in its later phases and then injected into recipients produced analgesic effects that differed little from those exerted by lumbar cord extracts. Comparison of the two PS elicited in this study suggests that the effects of substances isolated from the central nervous system of animals with a PS largely depend on how the GPEE is formed, i.e., whether it develops rapidly in an explosive manner, as in the PS of spinal origin, accompanied by a state of severe stress, or slowly and gradually over a prolonged period and to a relatively moderate degree, as in the PS induced by adjuvant arthritis. In the latter case, an important consideration is probably the weakening of adaptive mechanisms by which the animal is protected from the deleterious effects of pain.

In summary, the results of the present study indicate that substances of peptide nature possessing analgesic properties accumulate in the spinal cord of rats with a pain syndrome.

REFERENCES

1. T. V. Goryacheva, M. Yu. Karganov, E. I. Danilova, *et al.*, in: *Second All-Union Conference on Neurosciences. Abstracts of Papers* [in Russian], Kiev (1988), pp. 121-122.
2. G. N. Kryzhanovskii, *Determinant Structures in Nervous System Pathology* [in Russian], Moscow (1980).
3. G. N. Kryzhanovskii, M. Yu. Karganov, A.A. Shandra, *et al.*, *Byull. Eksp. Biol. Med.*, **107**, № 3, 271-274 (1989).
4. G. N. Kryzhanovskii, V.K. Lutsenko, and M.Yu. Karganov, *Ibid.*, **92**, № 10, 404-406 (1981).
5. G. N. Kryzhanovskii, V. K. Lutsenko, and M. Yu. Karganov, *Ibid.*, **93**, № 1, 14-16 (1982).
6. G. N. Kryzhanovskii, A. A. Shandra, and L. S. Godlevskii, *Ibid.*, **110**, № 7, 14-17 (1990).
7. G. N. Kryzhanovskii, A. A. Shandra, L. S. Godlevskii, *et al.*, *Usp. Fiziol. Nauk*, **23**, № 3, 53-77 (1992).
8. F. F. Bloom, *Science*, **245**, № 4, 114-121 (1981).
9. F. F. Bloom and J. Rossier, *Adv. Biochem. Psychopharmacol.*, **18**, 89-109 (1978).
10. C. Giurgea *et al.*, *Arch. Int. Pharmacodyn. Ther.*, **191**, 292-300 (1971).