PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Effects of Neurotropin in Pain Syndromes

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Neurotropin is shown to hinder the development of a neuropathic pain syndrome induced in rats by sciatic nerve transection and to produce a curative effect in animals that have already developed the pain syndrome. Neurotropin does not prevent the occurrence of adjuvant-induced arthritis in rats but significantly diminishes its manifestations. The observed effects of this agent are attributed to its action on different components of the pathological algetic system.

Key Words: *pathological algetic system; neuropathic pain syndrome; adjuvant arthritis*

At the basis of pain syndromes is a pathological algetic system (PAS) which is formed in the body and which consists of altered and interacting structures of the pain sensitivity system [9]. The clinical features of a pain syndrome depend on how the PAS is organized and activated and on how it functions. The neuropathic pain syndrome is characterized by increased excitability of peripheral nerve fibers [13] and of nociceptive neurons in the dorsal horns of the spinal cord, thalamic nuclei, and cerebral cortex [5]. In adjuvant arthritis, the sensitivity of peripheral high-threshold receptors and the level of neuronal activity in the superficial and deep areas of the spinal cord dorsal horns are elevated [10] and thalamic and cortical neurons become activated. In the treatment of a pain syndrome it is therefore desirable to use preparations that act on different components of the PAS. One such preparation is neurotropin - a nonprotein fraction extracted from subcutaneous inflammatory exudates of rabbits immunized with vaccina virus. The positive results gained with neurotropin in some pain syndromes (lumbago, cervicodynia, postherpetic neuralgia) led us to study its effects in the neuropathic pain syndrome and adjuvant arthritis.

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MATERIALS AND METHODS

A total of 60 male Wistar rats weighing 180-200 g were used, 10 rats in each group. A neuropathic pain syndrome was produced by dividing the left sciatic nerve under ether anesthesia at the level of the popliteal fossa and the proximal end of the nerve was placed in a sealed polyethylene tube and left in the wound, which was sutured. The severity of the pain syndrome was evaluated from the degree of autotomic damage to the denervated limb [8] as expressed in scores, and from changes in thresholds of pain sensitivity to thermal stimulation. Autotomic damage to 1, 2, 3, 4, and all claws was assigned scores 1, 2, 3, 4, and 5, respectively; damage to a phalanx of 1, 2, 3, 4, and 5 digits, scores 6, 7, 8, 9, and 10, respectively; and amputation of the limb at the level of the talocrural joint, score 11.

Adjuvant arthritis was produced by means of complete Freund's adjuvant consisting of lanolin (1 part), paraffin oil (30 parts), and a *Mycobacterium*

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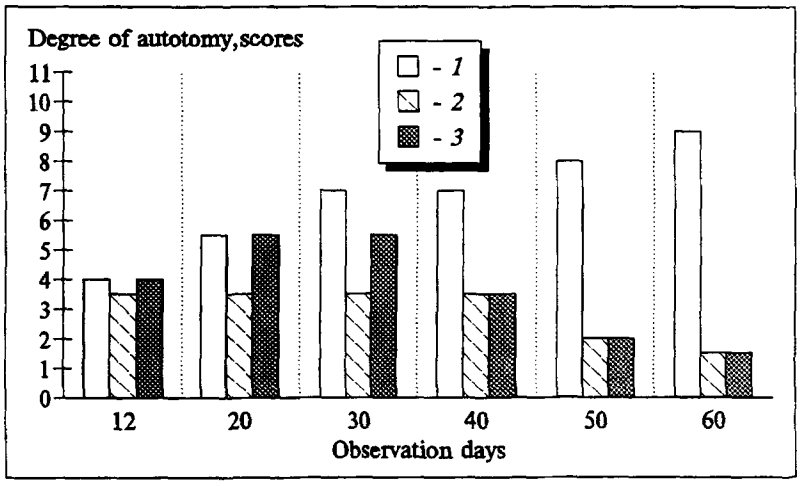

Fig. 1. Effect of neurotropin on the neuropathic pain syndrome. *1*) control group; *2*) group receiving neurotropin from the first postoperative day; *3*) group receiving neurotropin from the 12th postoperative day.

tuberculosis culture (2 mg/ml) inactivated by boiling for 90 min on three successive days. The adjuvant was injected into the left hind paw in an amount of 0.1 ml. The severity of the arthritis was assessed by the intensity of inflammatory changes in joints, and also by changes in body weight and in thresholds of pain sensitivity to thermal stimulation. The intensity of inflammatory changes was expressed in terms of a limb edema index defined by the formula: $(A\text{-}B)/B \times 100$, where A and B are the diameters (in mm) of the joint after and before injection of the adjuvant, respectively.

Pain sensitivity thresholds were measured in the hot plate test, for which the animals were placed on a metal plate heated to 55°C, and the time when they began to lick their hind paws was recorded in seconds.

Neurotropin (Nippon Zoki Pharmaceutical Co., Japan) was injected intraperitoneally in a dose of 1.0 ml once daily for 21 days starting on day 1 after sciatic nerve transection and on day 3 before adjuvant injection or on day 12 after sciatic nerve transection and on day 3 after adjuvant injection.

RESULTS

In the group that was not receiving neurotropin, autotomies were first seen 5-6 days after the nerve was cut: 1 or 2 rats out of 10 (in different test series) had bitten on one or two claws. On day 10-12, autotomies assigned score 4 were observed in 5 of the 10 rats (Fig. 1, *1*). By day 30, all claws and phalanges of one or two digits had been bitten off and interphalangeal joints were damaged in 7 rats. The animals were biting their paws in a paroxysmal manner and the attacks were preceded by clonic twitchings of the limb.

In the group where neurotropin treatment was started right after the operation, the first autotomies also appeared on days 5-6 (two claws were bitten off in 1 rat out of 10). By day 12, autotomies given score 3 were present in 3 rats, and they remained at this level on days 20, 30, and 40 (Fig. 1, *2*). Thereafter, the claws began to grow in these three rats and the pain syndrome gradually regressed. In this group, no further autotomies were observed right up to the end of the two-month observation period. Pain sensitivity thresholds, as measured in the hot plate test, remained unchanged throughout the observation period - unlike in the control group, where they became much lower (Table 1). In no case did the pain syndrome recur after the discontinuation of neurotropin treatment.

The group where neurotropin treatment was started on day 12 after sciatic nerve transection consisted of 10 rats in which the pain syndrome had reached a score of 4 by that day. In this group, despite neurotropin treatment, the severity of the pain syndrome by day 20 did not differ from that in the control group (Fig. 1, *3*); by day 40, however, claws started to grow, and the syndrome had a lower score (3 rather than 5 as on day 20). Pain sensitivity thresholds rose (Table 1). The pain syndrome recurred in 4 of the 10 rats 15 to 20 days after the termination of therapy, but it was less severe (score 3 at most).

On the day following injection of Freund's adjuvant, the affected paw became hyperemic and somewhat edematous. Three days later the edema increased (edema index of 35 as opposed to the normal value of 10). Ulcerations on the talocrural joint were present in 4 rats on day 20 and in all 10 rats on day 40 when the limb was very edematous (edema index 100); in 6 rats the other hind paw was also arthritic. The animals favored the affected limb when moving about, kept apart from each other, and displayed an aggressive reaction if

touched. When the affected paw was stimulated, they jerked it back and licked the damaged area.

In the group which started to receive neurotropin 3 days before adjuvant injection, arthritis developed just as in the control group during the first 5-7 days, the limb edema index being 40; by days 20 and 30, however, this index reached only 50 and 60, respectively, *vs.* 100 in the control group (Fig. 2). The animals were less aggressive when the affected paw was touched and tried to use the paw when walking. Although adjuvant arthritis did not extend to other limbs, hyperemia of the paw and ulcerations on the talocrural joint did not begin to diminish until day 40.

In the group of 10 rats where neurotropin treatment was started 3 days after adjuvant injection, the development of arthritis in the first 5-7 days differed little from that in the other two groups, the limb edema index being 45 on average; by day 20, this index dropped to 40, remained at this level till day 50, and then began to decrease (Fig. 2); 4 rats developed arthritis in the other hind limb, but the edema index did not exceed 30. Pain sensitivity thresholds rose in both neurotropin-treated groups (Table 1).

It is evident from the results presented above that neurotropin prevented the occurrence of autotomies in many animals with the neuropathic pain syndrome and also exerted therapeutic effects by reducing the severity of this syndrome when it had already developed. The mechanisms of action of neurotropin appear to be associated with its influence on neurotransmitter systems in the spinal cord. Acting at the supraspinal level, neurotropin inhibits through intermediate inhibitory systems, such as the serotoninergic, noradrenergic, and GABA-ergic systems, the release of pain transmitters (in particular substance P) at the spinal level [7], thereby making the dorsal horn neurons less excitable. The manifestations of denervation symptomatology in rats and rabbits are considerably intensified because of the incompetence of descending noradrenergic antinociceptive structures coupled with reduced activity of the GABA-ergic system. Neurotropin activates the noradrenergic system, as well as GABA-ergic neurons, by inhibiting GABA uptake [6,11]. The analgesic effect of neurotropin is also mediated by the serotonin system at the spinal and supraspinal levels [6]. It has been shown [4] that animals which do not exhibit autotomy over prolonged periods after denervation have high serotonin levels in the denervated spinal segments, which is associated with adaptive changes playing a protective role in preventing autotomy. Consequently, neurotropin may hinder the development of autotomy by enhancing the activity of the serotoninergic system at the supraspinal and spinal levels [7]. Neurotropin also contributes to enhanced activity of glycinergic transmission [1], and this, too, is conducive to lowering the level of autotomy, because blockage of the glycine receptors has the opposite effect [12]. Therefore, by acting on monoaminergic systems in spinal and supraspinal structures, neurotropin may prevent the development of a pathological algetic system or weaken its pathogenic activity in the neuropathic pain syndrome.

Neurotropin did not prevent the occurrence of adjuvant-induced arthritis but did inhibit its devel-

TABLE 1. Variation in Pain Sensitivity (as Assessed by the Reaction of Paw Licking, Sec) in Response to Neurotropin

Observation day	Neuropathic pain syndrome			Adjuvant arthritis		
	Control group	Neurotropin, 1.0 ml		Control group	Neurotropin, 1.0 ml	
		From day 12 of disease	From day 1 of disease		3 days before injection	3 days after onset of arthritis
0	9.0±0.4	9.1±0.5	9.0±0.5	9.2±0.5	9.4±0.6	9.1±0.5
3	9.1±0.5	9.1±0.5	8.9±0.4	8.9±0.3	8.0±0.3	7.8±0.3
7	8.4±0.5	8.9±0.6	8.7±0.4	7.3±0.5	8.3±0.5	8.2±0.5
10	7.8±0.6	9.3±0.4	7.7±0.5	7.9±0.3	10.3±0.5	11.0±0.7
17	6.8±0.5	9.4±0.6	13.6±0.6	7.1±0.3	16.1±0.3	15.2±0.6
24	6.1±0.4	9.4±0.4	19.1±0.6	6.9±0.5	15.4±0.5	14.9±0.3
30	6.0±0.4	9.3±0.6	17.8±0.4	7.4±0.4	12.1±0.6	11.7±0.7
38	7.2±0.6	9.1±0.5	13.9±0.2	8.1±0.6	11.3±0.4	11.0±0.3
45	7.1±0.7	8.9±0.5	13.4±0.3	8.9±0.3	10.6±0.4	10.2±0.4
50	7.3±0.9	9.0±0.3	10.2±0.5	9.3±0.4	9.0±0.7	9.2±0.6
60	8.0±0.8	9.1±0.4	9.2±0.4	9.0±0.6	9.2±0.6	9.0±0.5

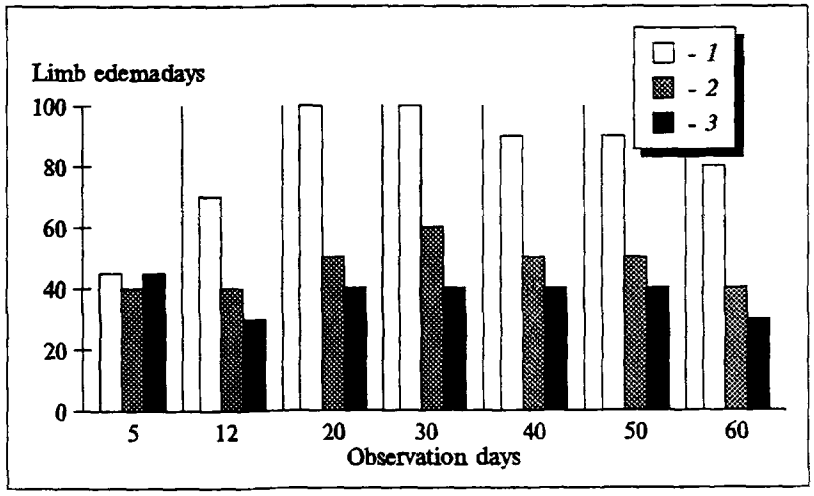

Fig. 2. Effect of neurotropin on adjuvant arthritis. *1*) control group; *2*) group given neurotropin 3 days before injection of Freund's adjuvant; *3*) group given neurotropin 3 days after adjuvant injection.

opment, irrespective of whether treatment was started before or after injection of the adjuvant. Pain arises in arthritis because of the activation and heightened sensitivity of fine peripheral fibers as a result of increased release of chemical agents. One of these is bradykinin, which increases the permeability of microvessels and thus creates excess pressure of interstitial fluid. Neurotropin blocks the release of bradykinin into the interstitial space, thereby reducing vascular permeability. At the basis of the spread of arthritis to the contralateral side lies a neurohumoral process in which chemical substances, in particular substance P, are released by fine terminals [3]. Probably, the ability of systemically administered neurotropin to inhibit the release of substance P explains why arthritis did not spread to the contralateral side in rats given this drug before adjuvant injection. An analgesic effect of neurotropin was demonstrated in the hot plate test by measuring the pain sensitivity threshold. These results agree with those of other authorities [2,7].

In summary, neurotropin exerts direct or indirect effects on various components of the pathological algetic system by acting on monoaminergic systems. In the neuropathic pain syndrome, neurotropin can prevent the formation or weaken the activity of the algetic system. In adjuvant arthritis it alleviates the latter by acting on tissue factors and altering vascular permeability.

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