DETECTION OF LATENT POSTURAL ASYMMETRY WITH THE AID OF A SYNTHETIC HEXAPEPTIDE

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The formation of a generator of pathologically enhanced excitation (PEE) [6] in the anterior horns of the spinal cord in the efferent outflow system has been shown to cause muscular rigidity of the corresponding hind limb [3, 5, 8]. This syndrome is manifested clinically as extensor rigidity because of hypertonia of the extensors. It follows from the theory of generator mechanisms of neuropathological syndromes [4, 6] that the neuropathological syndrome characterized by hyperactivity of the system is manifested if the PEE generator on which it is based reaches a certain level of power in its formation so that it can produce a sufficiently intensive functional volley to overcome inhibitory control and to activate effector structures. Much of the latent period of development of the syndrome is accounted for by the formation of the PEE generator. The question accordingly arises whether the power of the newly formed generator could not be increased by appropriate intervention and thus "brought to light" during the latent period. It follows from the same theory of generator mechanisms of neuropathological syndromes that abolition of the syndrome is linked with abolition of the generator and that the process of recovery takes place in stages, which correspond to the level of depression of generator activity [6]. Accordingly a second question arises: Cannot the generator be activated at a stage of recovery when it is sufficiently depressed for its activity to be no longer manifested clinically?

In both cases the hexapeptide Tyr-Gly-Gly-Phe-Leu-Arg, synthesized in the Laboratory of Peptide Synthesis (Director, Dr. Chem. Sci. M. I. Titov), All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, and which, as previous investigations have shown [2, 10], evokes postural asymmetry in animals, was used to activate the generator.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 200-220 g were used. Muscular rigidity of the hind limbs was induced by tetanus toxin (TT), which disturbs various types of inhibition in the spinal cord [3, 7, 11-13], and which thus induces the formation of a PEE generator from a population of disinhibited neurons [6]. TT in a dose of 0.025 MLD for rats with this body weight was injected into the muscles of the right and left leg and thigh. As was shown previously, TT travels along the regional nerves to the anterior horns of the corresponding segments of the spinal cord, where it induces the formation of a PEE generator [3, 6]. The hexapeptide was injected suboccipitally in a dose of 100 μg/kg in a volume of 0.05 ml, under brief ether anesthesia: in the experiments of series I 48 h after injection of TT, before any signs of increased extensor tone had appeared (the end of the latent period), in series II after disappearance of clinical signs of muscular rigidity (30 days after injection of TT). Animals into which the same peptide, but containing the D-stereoisomer of leucine, or physiological saline in the same volume, was injected served as the control. Clinical observations were made on the behavior of the animals, various stages of development of extensor tone were photographed, and electrical activity (EA) in the muscles was recorded by the RM-86M polygraph (from Nihon Kohden, Japan). In a preliminary series of experiments the hexapeptide was injected in the same dose into healthy animals (36 rats).

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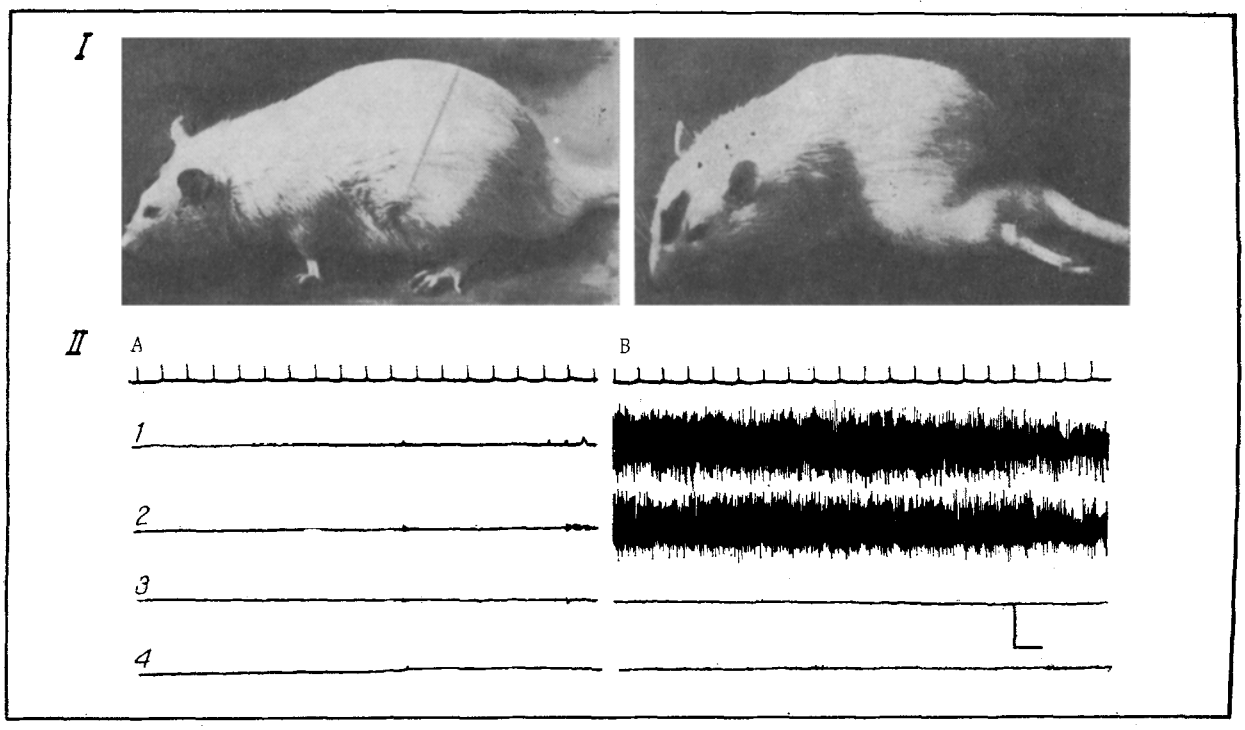

Fig. 1. Appearance of muscular hypertonia after suboccipital injection of peptide during latent period of development of the pathological process. IA) External appearance of animal 48 h after injection of TT (latent period) into left hind limb; IB) the same rat 5 min after injection of peptide in dose of 100 μg/kg; II) EA of hind limb muscles during latent period before (A) and 5 min after (B) injection of peptide. Scheme of derivations: 1) left gastrocnemius muscle, 2) posterior group of left thigh muscles, 3) right gastrocnemius muscle, 4) posterior group of right thigh muscles. Calibration: 250 μV, 1 sec.

EXPERIMENTAL RESULTS

The preliminary experiments showed that suboccipital injection of the hexapeptide induces postural asymmetry in healthy animals, manifested as the appearance of weak extension of the left or right hind limb, which persists for about 5 min. No definite preference for the effect to appear on the right or left side could be noted: Left-sided extension took place in 15 animals, right-sided in 12, and in nine animals it was difficult to decide on which side the effect was predominant. This result may be due to natural differences in muscle tone on the right and left sides; the effect of experimental conditions that were not taken into account (the mode of injection of the peptide, etc.) likewise cannot be ruled out.

In control experiments (injection of physiological saline or of a biologically inactive hexapeptide) no difference in muscle tone of the hind limbs was observed in any of the 15 rats.

Suboccipital injection of the hexapeptide 48 h after injection of TT and at the end of the latent period, when no sign of muscular hypotonia could be observed either visually (Fig. 1, IA) or electromyographically (Fig. 1, IIA) in the animals, distinct extension of the limb into which TT was injected was produced (Fig. 1, IB). This picture appeared immediately after the animals came round from the anesthetic. The rats moved about with the limb stretched out backward, and it took no part in walking. On passive flexion of the limb, resistance could be felt. Not until after 10-20 min did the animals begin to use the limb for moving. This effect was observed in every case (22 rats). The EMG showed a considerable increase in EA in the muscles of the left hind limb, in the form of a long, asynchronous discharge (Fig. 1, IIB). Since EA was recorded in unanesthetized animals, loosely fixed to the wall, frequent movements of the animals constantly provoked bursts of EA, which merged into uniformly enhanced EA. This type of EA is observed when the PEEE generator is located in the anterior horns [3, 6]. Enhanced EA persisted for 30-40 min. The effects described above was observed in all experiments (22 animals).

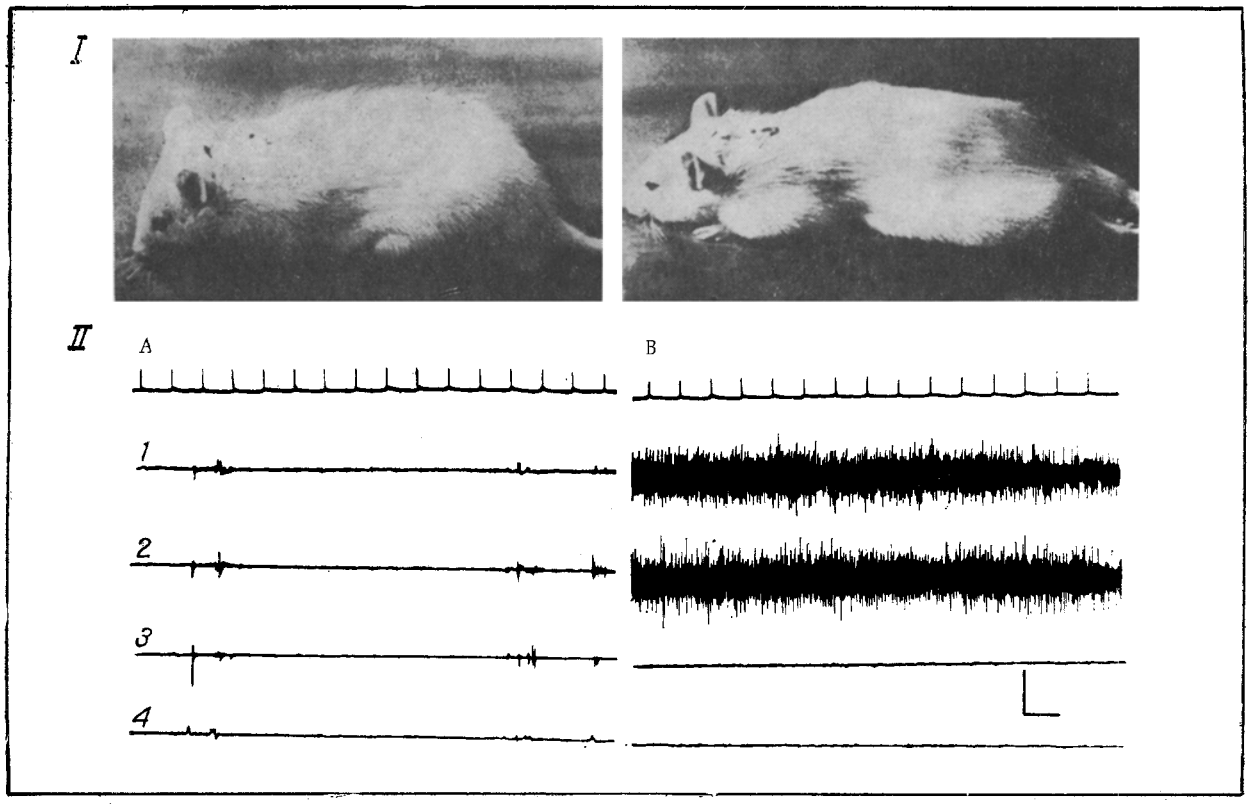

Fig. 2. Appearance of muscular hypertonia after suboccipital injection of peptide in animal during period of clinical recovery. IA) External appearance of animal 30 days after injection of TT into left hind limb (clinical recovery); IB) the same rat 5 min after suboccipital injection of peptide in dose of 100 μg/kg; II) EA of hind limb muscles in animal during clinical recovery period before (A) and 5 min after (B) injection of peptide.

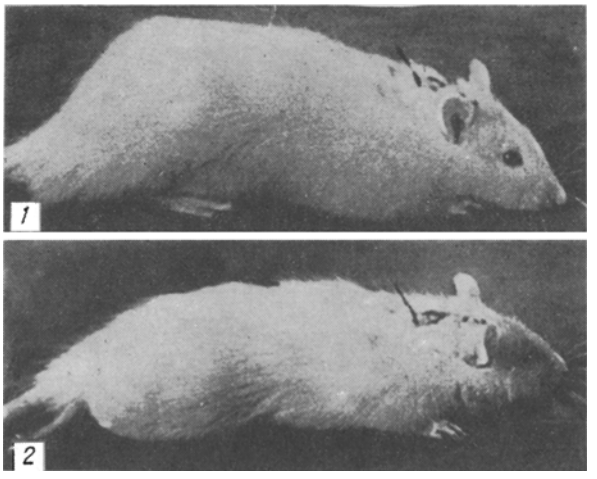

Fig. 3. Appearance of hypertonia of right hind limb muscles during period of clinical recovery after suboccipital injection of peptide. 1) Before, 2) 5 min after injection of peptide in dose of 100 μg/kg, 30 days after injection of TT into right hind limb muscles.

In the experiments of series II the peptide was injected in the stage of recovery, 30 days after injection of TT into the hind limb muscles, when no difference in muscle tone of the two hind limbs could be detected either clinically (Fig. 2, IA) or electromyographically (Fig. 2, IIA). Injection of the peptide into these animals induced a marked increase in muscle tone in the previously affected left hind limb, manifested as extension of the left hind limb (Fig. 2, IB) and as the appearance of characteristic EA in its muscle (Fig. 2, IIB, 1, 2). This type of effect was observed in 33 of the 35 animals.

In the control series of experiments no signs of postural asymmetry were observed in animals (17 rats) receiving physiological saline or the biologically inactive hexapeptide 48 h after injection of TT or in the stage of clinical recovery.

The appearance of muscular hypertonia and increased EA in the muscles after suboccipital injections of the hexapeptide into the recovered rats was independent of the side on which the PEE generator had previously been created: The same effect of an increase in muscle tone was obtained also in cases (in nine of nine animals) when an active generator had previously been located in the anterior horns of the lumbosacral segments on the right (Fig. 3).

These experiments showed that the hexapeptide which was used activates the PEE generator both in the stage of its formation and also in the stage of its clinical suppression; this effect, moreover, is independent of natural asymmetry of muscle tone. The increase in activity of the generator in the early stages of its formation may be due to potentiation of electrogenesis by the neurons of the generator and the recruiting of new neurons into it, inhibitory control over which is not yet completely disturbed. Similar mechanisms are involved also in activation of the suppressed generator in the stage of clinical recovery. This last case is interesting from several points of view. It shows, first, that the stage of clinical recovery is not equivalent to complete elimination of the pathological process (which calls for appropriate therapeutic tactics). The results are also evidence of the importance of trace reactions in the pathology of the CNS [6, 8] and they can also explain phenomena such as recurrences, the locus minoris resistentiae phenomenon, and differences in the course of a new pathological process after recovery from an illness, described in the past as the "second blow." These results are particularly interesting in the context of the study of memory mechanisms. They show that these mechanisms in principle exist at the spinal level. A PEE generator can be used as model of certain forms of memory, and to study it [6]. This conclusion is in agreement with a similar appraisal of the epileptic focus [1]. As has already been pointed out [6], an epileptic focus in the cerebral cortex is one form of PEE generator.

The effect of appearance of asymmetry of muscle tone in the hind limbs of animals which have recovered also has been observed after systemic administration of phenol [3, 9], which is known to act mainly on spinal motoneurons. However, the effect of phenol is weaker and arises against the background of general clinical spasms of all muscles. The fact that the hexapeptide containing the D-amino acid does not induce this phenomenon is evidence that the effect possesses some degree of specificity. A connecting link is that the biologically active hexapeptide used in the present experiments affects regulation of muscle tone, as experiments on healthy animals in the course of the present investigation and undertaken by other workers have shown [2, 10]. These data are in agreement with the general principle that in the early stages of formation of the generator, it is activated by stimuli of a particular modality, specific for the system in which the generator appeared [6].

LITERATURE CITED

1. I. P. Ashmarin, M. Yu. Eropkin, T. A. Kovaleva, et al., Mol. Biol., No. 5, 965 (1978).
2. I. P. Ashmarin, in: The Theoretical Basis of Pathological States [in Russian], Leningrad (1980), pp. 168-172.
3. N. P. Bekhtereva, The Healthy and Sick Human Brain [in Russian], Leningrad (1980).
4. G. A. Vartanyan and Yu. V. Balabanov, Byull. Éksp. Biol. Med., No. 8, 147 (1978).
5. G. N. Kryzhanovskii, Tetanus [in Russian], Moscow (1968).
6. G. N. Kryzhanovskii, Vestn. Akad. Med. Nauk SSSR, No. 11, 42 (1979).
7. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
8. G. N. Kryzhanovskii, V. N. Grafova, and E. I. Danilova, Byull. Éksp. Biol. Med., No. 5, 515 (1977).
9. G. N. Kryzhanovskii, V. N. Grafova, and E. I. Danilova, Byull. Éksp. Biol. Med. (1981).
10. Yu. S. Sverdlov, Neirofiziologiya, No. 1, 25 (1969).
11. G. Ungar, Fiziol. Cheloveka, No. 3, 808 (1977).
12. E. I. Chazov, V. N. Smirnov, M. I. Titov, et al., Vestn. Akad. Med. Nauk SSSR, No. 7, 50 (1980).
13. J. C. Eccles, The Physiology of Synapses, Berlin, Springer (1964).

14. V. B. Brooks, D. R. Curtis, and J. C. Eccles, J. Physiol. (London), 135, 655 (1957).
15. T. R. Curtis and A. R. Johnston, Rev. Physiol. Biochem. Pharmacol., 69, 97 (1974).
16. D. De Wied, A. Witter, and H. M. Greven, Biochem. Pharmacol., 24, 1463 (1975).
17. D. De Wied and W. H. Gispen, in: Peptides in Neurobiology, New York (1977), pp. 397-448.
18. T. B. van Wimersma Greidanus, in: Central Nervous System Effects of Hypothalamic Hormones and Other Peptides, New York (1979), pp. 177-187.