AFFERENT DETERMINATION OF TRANSFER FACTOR FORMATION IN THE RAT SPINAL CORD

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Unilateral destruction of regions of the cerebellum [2], cortex [9], and Deiters' nucleus [10] in animals induces asymmetry of muscle tone, i.e., gives rise to symptoms of postural asymmetry. These symptoms can be induced in intact recipients by intracerebral injection of an extract of the brain or CSF from a donor with postural asymmetry. Substances from the donor's brain inducing the appearance of the corresponding symptoms in the recipients has been called postural asymmetry factor or transfer factors (TF) [5]. In biochemical investigations, TF have been identified as peptides with a molecular weight of 1-2 kilodaltons [2, 9, 10]. The biological role of TF, and the source and mechanisms of their formation in the donor's brain, as well as their "point" of application and method of interaction with neurons in the recipient's brain remain unknown. The view is held that TF are a component of the mechanism of formation of a stable pathological state, such as arises immediately after injury to part of the brain [3, 4]. The view also is held that TF are substances responsible for the lateralization of brain function [11] and are present in the normal (uninjured) brain [8]. To determine the biological role of TF it is important to investigate the causes determining their appearance in brain tissue. In experimental injury to a part of the brain axonal transport is disturbed and the afferent inflow is modified to the zones which were connected with the region of injury. It is these factors which may be the causes initiating TF formation in the brain [1]. The possible role of the factor of injury to the integrity of neurons and their processes as the immediate cause of TF formation must also be taken into consideration.

The aim of this investigation was to discover the leading cause determining the appearance of TF in the brain.

EXPERIMENTAL METHOD

The spinal cord–nerve–muscle system was chosen as the experimental model, for it enabled controlled denervation and deafferentation procedures to be carried out without disturbing the integrity of the neuron net. Experiments were conducted on noninbred albino rats weighing 180-200 g. In donor rats of group 1 (n = 50) the left tibial nerve, supplying the posterior group of calf muscles of the left limb, was divided 48 h before removal of the spinal cord. To differentiate between the effect of nerve injury and deafferentation on TF formation, the animals of group 2 (n = 50) were given two injections, with an interval of 24 h between them, of a mixture consisting of 2% procaine and 70% ethyl alcohol (1:1), in a volume of 0.5 ml, into the posterior group of muscles of the left calf. Preliminary experiments showed that the time of 50% recovery of the threshold of response to direct electrical stimulation of the muscles after a single application of alcohol–procaine blockade was not less than 45-50 h. In the rats of group 3 (n = 50), in order to differentiate between the effect of alcohol–procaine deafferentation and possible disturbances of axonal transport on TF formation, the spinal cord was removed 2 h ± 15 min after injection of the alcohol–procaine mixture. The possibility of differentiation in this way is based on the fact that at the upper limit of velocity of the axonal flow of not more than 500 mm/day [6], and with the length of the nerve being not less than 52 mm, the shortest time of possible biochemical changes in the spinal cord must be 2.5 h, but the aftereffects of deafferentation arises much earlier, for the afferent inflow was blocked 3-5 min after the alcohol–procaine blockade. Intact rats (n = 50) constituted group 4. The spinal cord extract was obtained from the donor rats by the method in [9]. Evidence that the spinal cord extract contained TF was given by the results

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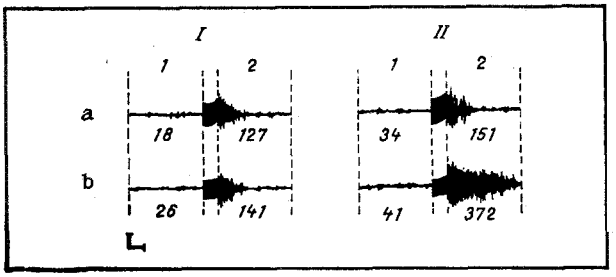

Fig. 1. EMG responses of right (a) and left (b) gastrocnemius muscles of recipient animal before (I) and after (II) injection of spinal cord extract from donor with divided left tibial nerve. Numbers below EMG traces indicate number of EMG pulses which exceed the 25 μV threshold in the course of 5 sec before (1) and after (2) electrical stimulation. Numerical calculations of coefficients of change of responses: I) $P_{b.R} = 109$; $P_{b.L} = 115$; $K_R = \frac{117-109}{109} = 0.073$; II) $P_{a.R} = 117$; $P_{a.L} = 331$; $K_L = \frac{331-115}{115} = 1.878$; (R — right, L — left, a — after, b — before). Calibration: 1 sec, 300 μV.

of a biological test, revealing asymmetry of reflex EMG responses of the gastrocnemius muscle in the recipient rats. Spinal rats (division of the spinal cord in the upper thoracic region) were used as recipients, and were given a subdural injection of 30 mg of the extract (2.5 mg protein), diluted in 0.1 ml distilled water (30 mg is the mean quantity of dry extract obtained from the spinal cord of one rat) through an injection needle at the level L_3-L_5. The dilution volume was chosen in view of the need to obtain a near-physiological concentration of the salt present in the spinal cord extract. The EMG was recorded by bipolar needle electrodes (area 1 mm^2, interelectrode distance 10 mm). The strength of the stimulus was 3 thresholds of the direct response of the muscle. Preliminary experiments showed that the intensity of the EMG reflex response in the spinal animals was maximal when the parameters of electrical stimulation of the muscles were as follows: stimuli 0.5 msec in duration (1 kHz), in groups of 30, in 15 bursts, with an interval of 30 msec between bursts. The EMG reflex response was assessed by counting the number of crossings of the threshold, which was established at 25 μV (with a noise level of about 10 μV). Analysis of the response began 50 msec after the end of stimulation and continued for 5 sec. Stimulation was applied on each side 12 times: maximal and minimal responses were excluded and the remaining 10 were averaged. The interval between stimuli was 1 min. EMG responses were compared with spontaneous activity during the preceding 5 sec. The averaged difference between the number of EMG discharges after and before stimulation served as the measure of the response. The response was assessed during stimulation on the two sides alternately, both before and 45-60 min after injection of the extract of the recipient's spinal cord. Since all denervation and deafferentation manipulations on the donors were carried out on the same (left) side, the criterion of the presence of TF in the spinal cord extract was significant asymmetry of reflex EMG responses in the recipients after receiving an injection of the extract, compared with the state before injection. Because of some degree of asymmetry of the EMG responses observed before injection of the extract, the difference between the responses before and after injection of the extract was normalized for the value of the response before injection of the extract:

$$K = \frac{P_a - P_b}{P_b},$$

where P_a is the EMG response of the recipients after the injection, P_b is the same before injection of the spinal cord extract, and K the coefficient of change of the response. The significance of differences between the coefficients of the change in responses on the right and left sides was determined from data obtained on 12-17 recipient animals, and calculated by Wilcoxon's test for tied pairs [7]. An example of one of these experiments is given in Fig. 1 and shows the order of calculation of the coefficient of change of the response.

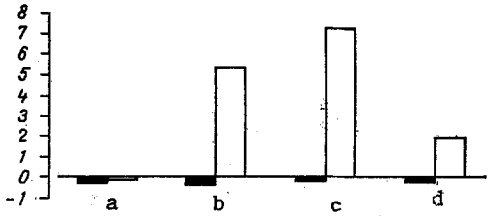

Fig. 2. Averaged value of coefficients of change in muscle responses of recipient rats to direct electrical stimulation of gastrocnemius muscles after injection of extract of donors' spinal cord. Ordinate, coefficient of change of response. a) With injection of spinal cord extract of intact rats; b) of rats with divided tibial nerve; c, d) 48 and 2 h respectively after alcohol–procaine block of the posterior group of left calf muscles. Black column – right, unshaded column – left limb. a) $p \gg 0.05$; b, d) $p < 0.05$; c) $p < 0.01$.

EXPERIMENTAL RESULTS

After injection of the spinal cord extract from the control (intact) rats into the recipient rats, as a rule some reduction of the EMG responses was observed on both sides. No asymmetrical changes were found in the responses of the muscles (Fig. 2a).

The results of testing the spinal cord extract from rats with a divided left tibial nerve differed significantly from the control (Fig. 2b). On the left side the EMG responses were increased compared with the state before injection of the extract, whereas on the right side they were unchanged or somewhat weakened. The significance of differences between the coefficients on the right and left sides was not less than 95%, evidence of the presence of TF in the spinal cord extract tested.

Injection of spinal cord extract from rats 48 h after alcohol–procaine blocking of muscle afferents into the recipients gave rise to a picture similar to that observed after injection of spinal cord extracts from rats with a divided left tibial nerve: enhancement of EMG responses on the left side and little change (most frequently reduction) of responses on the right side. The significance of differences between the EMG responses on the two sides was not less than 99% (Fig. 2c).

Averaged results of biological testing of spinal cord extracts of rats 2 h ± 15 min after alcohol–procaine blockade of the posterior group of left calf muscles before removal of the spinal cord are given in Fig. 2d. EMG responses of muscles on the left side of the recipient rats were greater than those on the right (significance not less than 95%).

LITERATURE CITED

1. E. I. Varlinskaya, G. P. Obukhova, and G. A. Vartanyan, Byull. Éksp. Biol. Med., No. 3, 259 (1984).
2. G. A. Vartanyan and Yu. V. Balabanov, Byull. Éksp. Biol. Med., No. 8, 147 (1978).
3. G. A. Vartanyan, Fiziol. Cheloveka, 7, 474 (1981).
4. G. A. Vartanyan and B. I. Klement'ev, Fiziol. Cheloveka, 9, No. 1, 122 (1983).
5. G. A. Vartanyan and M. I. Lokhov, Principles and Mechanisms of Activity of the Human Brain [in Russian], Leningrad (1985), pp. 34-36.
6. R. N. Glebov and G. N. Kryzhanovskii, Functional Biochemistry of Synapses [in Russian], Moscow (1978).
7. G. N. Zaitsev, Methods of Biometric Calculation [in Russian], Moscow (1973).
8. M. Yu. Karganov, Patol. Fiziol., No. 5, 93 (1981).
9. B. I. Klement'ev, M. A. Danilovskii, and I. P. Shul'gina, Proceedings of the 4th Soviet–Italian Symposium on Neuropsychopharmacology [in Russian], Moscow (1981), pp. 25-26.
10. G. N. Kryzhanovskii, V. K. Lutsenko, M. Yu. Karganov, and S. V. Belyaev, Patol. Fiziol., No. 3, 68 (1984).
11. V. K. Lutsenko and M. Yu. Karganov, Neirokhimiya, 4, No. 2, 197 (1985).
12. J. Hariga, "Influences sur la motricité de la suppression des effeteurs gamma par alcolisiation des nerfs périphériques," Thesis, Paris (1966).
13. R. Miledi and C. R. Slater, J. Physiol. (London), 207, 507 (1970).