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# ACTIVITY OF POSTURAL ASYMMETRY FACTORS IN SYMMETRICAL REGIONS OF THE RAT SPINAL CORD

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UDC 616.832-008.6-092.9

KEY WORDS: postural asymmetry factor; lumbar enlargement of the spinal cord; unilateral injury to the neocortex

Interest in the problem of chemical asymmetry of the brain continues to increase in connection with the study of functional asymmetries which are characteristic of the normal and injured CNS [10]. Comparatively recently a new class of endogenous oligopeptide factors inducing an asymmetrical distribution of tone of the limb muscles in spinalized and intact animals, leading to the formation of postural asymmetry (PA), has been discovered. Postural asymmetry factors (PAF) were first found in association with unilateral injuries of central motor systems [2, 3, 7]. Later they were found in the cerebral hemispheres [1, 8] and also in tissues of the spinal cord [9] and pituitary gland [11] of intact animals. On the basis of these data it was suggested that there exist neuropeptide modulators of a system regulating the state of muscle tone of the limbs under normal conditions and in association with unilateral injury to the CNS [4].

This paper describes a study of the distribution of PAF activity in symmetrical regions of the spinal cord of the intact animal, and also of animals with unilateral injury to the motor area of the neocortex.

## EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g were used. Under ether anesthesia the representation of the right hind limb in the motor area of the neocortex of the left hemisphere was removed. The animals were decapitated 48 h later, the lumbar enlargement of the spinal cord was removed and divided into right and left halves, taking bearings from the median sulci of the dorsal and ventral surfaces. The material was extracted with 0.2 N HCl by the method described previously [6]. The lyophilized extract was dissolved in 0.1% TFA and fractionated by gel-filtration on a column measuring 5 ml (0.6 × 20 cm), filled with Sephadex G-25 (superfine), in 0.1% TFA. The eluate was divided into two fractions: high-molecular-weight (over kD, $V_o$) and low-molecular-weight (under 2 kD, $V_e$), which were neutralized with 1 M $NH_4OH$ and lyophilized. An aqueous solution of the test material in a volume of 10 µl was injected intracisternally into a group of intact recipients, consisting of 10-12 rats. PA was recorded by the method in [6]. Material was considered to be active if, after injection, the fraction of animals with flexion of one hind limb, the right for example, was significantly larger than the fraction of animals with flexion of the left limb. In that case the PAF was called right-sided. PAF inducing flexion of the left limb was called left-sided PAF. The significance of differences was determined statistically by Fisher's exact method [5]. Activity of PAF was assessed as the number of minimal active doses per milligram of tissue.

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0007-4888/89/0004-0447$12.50 

TABLE 1. Inactivation of Low-Molecular-Weight PAF (fraction $V_e$) by High-Molecular-Weight Components (fraction $V_o$) of Extracts of Lumbar Enlargement of Spinal Cord

| Source of isolation of fractions | Ratio between activities of low- and high-molecular-weight PAF in mixture $V_e:V_o$ | Number of recipients | Number of recipients with flexion | | Activity of PAF in mixture |
|---|---|---|---|---|---|
| | | | right | left | |
| Intact animals | | | | | |
| right half of enlargement | 1:1 | 12 | 1 | 1 | — |
| left half of enlargement | 1:1 | 11 | 3 | 2 | — |
| Animals with injury to neocortex | | | | | |
| right half of enlargement | 10:1 | 11 | 1 | 1 | — |
| left half of enlargement | 10:1 | 11 | 4 | 1 | — |

Legend. —) Absence of significant excess of fraction of recipient animals with right-sided or left-sided flexion ($p > 0.05$).

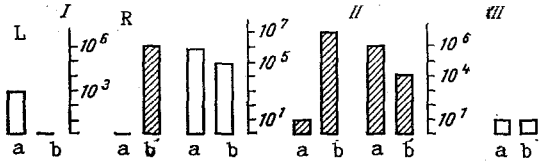


Fig. 1. Activity of PAF in unfractionated extract (I) and also in low-molecular-weight (II) and high-molecular-weight (III) fractions of left (L) and right (R) halves of lumbar enlargements of spinal cord of intact animals (a) and animals with injury to motor area of neocortex of left hemisphere (b). Unshaded columns — activity of PAF inducing left-sided flexion; shaded columns — activity of PAF inducing right-sided flexion. Ordinate, level of activity (number of minimal active doses per milligram of tissue.

## EXPERIMENTAL RESULTS

The study of extracts of halves of the lumbar enlargement of intact animals revealed only left-sided PAF, which was located in the left half of the spinal cord (Fig. 1). However, after fractionation of the extracts on Sephadex it was found that each half of the lumbar enlargement contained both high- and low-molecular-weight PAF. The low-molecular-weight factors were able to induce ipsilateral, and the high-molecular-weight factors — contralateral flexion relative to the half of the spinal cord (right or left) from which they were isolated. The results indicate the existence of a two-component system of chemical regulation of muscle tone at the segmental level, consisting of high- and low-molecular-weight PAF. It is important to note that within each half of the spinal cord tested this system was represented by factors inducing contralateral flexor responses. It will be clear from Fig. 1 that in the normal animal activity of the high- and low-molecular-weight factors in each half of the lumbar enlargement was comparable. This fact, considering the contralateral effect of high- and low-molecular-weight PAF, is evidently the cause of their mutual inactivation in unfractionated extracts. The mutually neutralizing effect of high- and low-molecular-weight PAF, with opposite sides of action, was demonstrated by testing mixtures consisting of aliquots of the $V_o$ and $V_e$ fractions, equal in absolute values of activity of the PAF contained in them (Table 1). It will be clear from Table 1 that this combination

makes the mixture unable to induce PA in the recipients. Thus under normal conditions muscle tone of the hind limbs is regulated by high- and low-molecular-weight PAF which are contralateral as regards their side of action.

After destruction of the left motor cortex, only right-sided PAF was found in unfractionated extracts of halves of the lumbar enlargement, and it was located in the right half of the enlargement (Fig. 1). Just as in the normal animal, low-molecular-weight PAF with ipsilateral and high-molecular-weight PAF with contralateral action were discovered in both halves of the spinal cord by gel-filtration. A characteristic feature of the state of partial supraspinal deafferentation of the right half of the lumbar enlargement is the development of an imbalance in the activity of the PAF contained in it. This was expressed as a marked increase in activity of the low-molecular-weight PAF (by $10^6$ times) compared with normal, whereas activity of the high-molecular-weight factor remains unchanged.

An imbalance also developed between high- and left-molecular-weight PAF in the left half of the enlargement, but due to an unequal decrease in their activity. However, although as a result of these changes activity of the low-molecular-weight (left-sided) factor was an order of magnitude higher than activity of the high-molecular-weight (right-sided) factor, the unfractionated extract of the left half of the spinal cord did not induce PA when tested biologically (Fig. 1). It remains to suggest that in this case the action of the low-molecular-weight PAF was neutralized by high-molecular-weight components of the extract, including those which were not high-molecular-weight PAF. In fact, the high-molecular-weight fraction obtained from extract of the left half of the lumbar enlargement inactivated low-molecular-weight PAF even when the activity of the high-molecular-weight right-sided PAF in it was only one-tenth of the activity of the left-sided PAF in the low-molecular-weight fraction of this same extract (Table 1). It will be clear from Table 1 that the $V_o$ fraction isolated from extract of the right half of the lumbar enlargement also possessed a similar property. Consequently, even in the early post-traumatic period, high-molecular-weight compounds described previously as inactivation factors (IF) of oligopeptide PAF appear in the spinal cord [6]. In the work cited IF were identified as high-molecular-weight components of rat brain extract, completely inactivating right-sided oligopeptide PAF toward the end of the 3rd week after injury to the motor cortex of the left hemisphere, at the stage of recovery of postural symmetry of the limbs.

What is the biological role of the appearance of this IF in the acute post-traumatic period? To answer this question, we shall start from views on the important role of oligopeptide PAF in the induction of compensatory reorganizations aimed ultimately at restoring the symmetrical distribution of limb muscle tone after unilateral injury to the neocortex [4]. According to these views, the normal course of the recovery process in the acute post-traumatic period is accompanied by activation of oligopeptide PAF, with affinity for the partially deafferented half of the spinal cord. One such PAF in this case is the low-molecular-weight right-sided PAF whose activity in the right hemisegments of the lumbar enlargement rises sharply compared with the normal state. The early appearance of IF in this zone may perhaps be evidence of the initial stages of the compensatory process, while the IF level is still too low for complete inactivation of the low-molecular-weight right-sided PAF, as is the case in the stage of recovery of postural symmetry of the limbs. In the left half of the spinal cord, with intact corticospinal connections, activity of the high-molecular-weight components completely neutralizes the action of the low-molecular-weight left-sided PAF contained there. Thus processes of activation of the right-sided and inactivation of the left-sided low-molecular-weight PAF are combined in the genesis of postural asymmetry after injury to the motor area of the neocortex of the left hemisphere.

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# RESTORATION OF MYOCARDIAL CONTRACTILITY DURING GRADUAL REPERFUSION AFTER TOTAL ISCHEMIA

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UDC 616.127-005.4-003.93-07: 616.127-008.6]-092.9

KEY WORDS: heart; ischemia; reperfusion; purine bases.

Restoration of myocardial contractility during reperfusion depends not only on the reversibility of the disturbances induced by ischemia, but also on the damaging action of reperfusion itself on metabolism, ultrastructure, and function [8, 10]. The principal factors damaging the myocardium during reperfusion are excessive reoxygenation and accumulation of cytoplasmic calcium [6]. The addition of antioxidants and calcium antagonists to the reperfusion solution, therefore, improves the restoration of the contractile function and metabolism of the ischemic myocardium [4, 7, 9]. Another approach was used in the present investigation, conducted on isolated guinea pigs' hearts, namely gradual restoration of the reperfusion rate to its initial level. Two versions of gradual reperfusion were used: circulating, when the perfusion fluid passed once through the heart, and recirculating, when the perfusion fluid passed through the coronary vessels several times.

## EXPERIMENTAL METHOD

Experiments were carried out on isolated hearts of guinea pigs weighing 200-300 g, anesthetized with urethane (1.25-1.50 g/kg). The heart was perfused in the retrograde direction by Langendorff's method with Krebs' solution (37°C) in a constant volume velocity of 10-12 ml/min/g. A small latex balloon, filled with liquid, was introduced into the left ventricle. The pressure in the left ventricle, and also the perfusion pressure in the aorta, were recorded by means of Gould Statham P23Db strain-guage transducers on a Gould Brush 2200 instrument. The contractile function was calculated as the product of the developed pressure and heart rate (HR), and the coronary resistance (CR) as the ratio of perfusion pressure to specific volume velocity perfusion.

After a period of stabilization of the contractile function (20-30 min) perfusion of the heart was completely stopped for 25 min, creating total ischemia, and this was followed by reperfusion for 30 min. In the control series (n = 12) reperfusion was carried out with the initial volume velocity. In the series of gradual circulating reperfusion (n = 8) the the initial volume velocity was 13 ± 1% of the initial value, and it increased by the same amount every 4 min of reperfusion, to reach the initial value by the end of reperfusion. In

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 0007-4888/89/0004-0450$12.50 