ASYMMETRY OF DISTRIBUTION OF PEPTIDE MUSCLE TONE REGULATORS AND SUBSTANCE P IN THE SPINAL CORD OF RATS WITH UNILATERAL HYPERACTIVITY OF LUMBAR ENLARGEMENT NEURONS

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UDC 616.74-009.1-02:616.832-008.94:577.175.82/-092.9

KEY WORDS: muscle tone; substance P; peptides; asymmetry.

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Persistent asymmetry of muscle tone of the hind limbs arises in rats with vestibulopathy [2] and it lasts even after abolition of descending influences by cordotomy (spinal memory) [4]. Injection of peptide factors present in the lumbar enlargement of the spinal cord of rats at this stage of vestibulopathy into intact recipients causes changes in muscle tone of the hind limbs similar to those observed in the donors [3]. These facts are evidence of the important role of peptides in maintenance of the asymmetrical change of spinal cord function in vestibulopathy. It might be supposed that this effect would be observed in other forms of unilateral motor hyperactivity of the spinal cord. It must be based on a relatively stable change in the peptide regulators of muscle tone in the left and right halves of the spinal cord.

These hypotheses were tested in experiments on animals in which asymmetry of muscle tone of the hind limbs was induced by the creation of a generator of pathologically enhanced excitation (GPEE) with the aid of tetanus toxin (TT) in the lumbar segments on one side of the spinal cord. Under these conditions considerable and relatively constant unilateral hyperactivity of the lumbar enlargement neurons appeared. Changes in peptide distribution in the spinal cord were judged by the intensity of the asymmetrical responses of the hind limbs in intact recipients, injected with peptide factors extracted from the whole spinal cord of rats with the GPEE and from its separate halves. The concentration of substance P (SP) in the spinal cord on the left and right sides of the midline also was investigated. We know that SP induces tonic discharges of motorneurons through its action on the rat spinal cord [13].

EXPERIMENTAL METHOD

A preparation of TT from Perm' Research Institute of Vaccines and Sera, purified by gel-filtration on Sephadex G-100 was used to create a GPEE in the ventral part of the spinal cord [2]. The purified toxin, in a dose of 4 MLD (for rats) and in a volume of 0.2 ml, was injected into the gastrocnemius muscle of rats weighing 200-220 g, on the right or left side. The spread of the toxin through the blood was blocked by injecting antitetanus serum (0.025 IU/0.2 ml) into the femoral or caudal vein. Traveling along the regional neural pathway [1], the TT first disturbed inhibitory mechanisms in the efferent outflow system, as a result of which a GPEE appeared in this situation, where it was manifested as increased electrical activity and rigidity of the gastrocnemius muscle. As the TT spread in the spinal cord, on the 4th-5th day a GPEE appeared in the system of regional propriospinal interneurons [2, 6]. Simulation of the "tetanic" limb induced activation of this GPEE, as shown by generalized convolutions: similar stimulation, applied to the sound limb, did not induce convulsions – merely the ordinary response of limb withdrawal. This phenomenon was described as the "universal despatch station phenomenon" or spinal myoclonus [2].

When this phenomenon appeared the animals were killed, tissue from the spinal cord and brain was removed, and the material was then extracted and changes in muscle tone of the hind

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 12, pp. 657-660, December, 1987. Original article submitted July 21, 1986.

0007-4888/87/0012-1655$12.50

TABLE 1. Duration (in sec) of PE of Hind Limbs of Healthy Rats after Intracisternal Injection of Extracts of the Lumbar Enlargement of the Spinal Cord in Whose Ventral Roots a Unilateral GPEE Had Been Formed (M ± m)

Donor animal	Extract	Number of animals		Hind limb	
		total	with PE	left	right
Healthy rats	Of spinal cord	10	5	0,7±0,6	2,4±0,8
Rats with GPEE:					
In right half of lumbar enlargement	Of brain	10	2	0,8±0,8	1,5±1,5
	Of spinal cord	10	10	0	18,5±1,7*
	Of spinal cord after proteolysis	10	1	0	0,8±0,8
	Of spinal cord + naloxone	10	3	0	2,5±1,4
In left half of lumbar enlargement	Of spinal cord	15	13	13,1±3,4*	0,3±0,3
	Of spinal cord after proteolysis	10	2	0,9±0,7	0
	Of spinal cord + naloxone	10	2	1,8±0,7	0

Legend. *$p_t < 0.01$ compared with corresponding control.

limbs induced by it were investigated as described previously [3]. Solutions of the extracts were injected intracisternally in a dose of 5 μg (protein) in 10 μl of solution.

Determination of SP by immunoassay was carried out with a kit of reagents from Amersham International (England). Tissue from each half of the spinal cord of the control and experimental animals was homogenized separately in 0.5 ml of buffer of the following composition: 50 mM Na_2HPO_4, 10 mM EDTA, 0.3% bovine serum albumin (Serva, West Germany), pH 7.2. Samples measuring 0.8 ml contained 500 μl of buffer, 100 μl of ^{125}I-SP with activity of 36,000 cpm, 100 μl of antiserum to SP, and 100 μl of the test extract. Polyethylene tubes with the samples were allowed to stand for 24 h at 4°C, after which each sample was treated with 0.5 ml of a freshly prepared suspension of activated charcoal (1 g charcoal in 50 ml of buffer), followed by vigorous shaking for 20 min. Charcoal was removed by centrifugation of the tubes in a "Rotamix" centrifuge (LKB, Sweden) at 4500 rpm for 15 min. Radioactivity of the residue was measured on a gamma-counter (LKB). The SP concentration was determined from a calibration curve plotted with unlabeled SP (Serva), and the protein concentration in the extract was determined by Bradford's method [8].

EXPERIMENTAL RESULTS

The creation of a unilateral GPEE in the system of propriospinal neurons located in the ventral half of the lumbar division of the spinal cord led to the appearance of ability to induce asymmetrical changes in muscle tone of the recipients' hind limbs in tissue extracts from this portion of the spinal cord: an increase in the duration of passive extension (PE) of the recipient's hind limb took place on the side on which the GPEE was formed in the donor. Incidentally, the appearance of lateralization factors (LF) of muscle tone was observed only in the spinal cord. Brain extracts did not induce asymmetrical effects (Table 1). Incubation of the spinal cord extracts with pronase, leading to proteolysis of the peptide fraction, completely abolished the ability of the extracts to induce lateralized changes of muscle tone in the intact recipients (Table 1). The same result, namely suppression of the action of the extracts, was obtained by the use of naloxone, a blocker of opiate receptors, injected together with the extract in a dose of 0.2 μg (Table 1).

Extracts of halves of the spinal cord of healthy rats, unlike extracts of the whole brain, caused changes in muscle tone of the ipsilateral hind limbs (Table 2). After the appearance of a unilateral GPEE the effectiveness of the extracts of halves of the spinal cord underwent significant changes: on the side of the GPEE it increased, on the opposite side it decreased (Table 2).

A study of the EP concentration in the left and right halves of the spinal cord of healthy rats did not reveal any asymmetry of SP distribution (Table 3). The creation of a right-side GPEE in the ventral half of the spinal cord led to a significant increase in the SP concentration in each half; moreover, on the side of the GPEE the increase in SP concentration was more marked (five times greater than in the control) ($p_t < 0.01$).

TABLE 2. Duration (in sec) of PE after Injection of Extracts of Right and Left Halves of Spinal Cord of Healthy Animals and of Rats with GPEE (M ± m)

Extract injected	Number of animals		Hind limb		p_t
	total	with PE	right	left	
Of right half					
Healthy rats	10	10	18,6±4,3	2,5±1,1	<0,01
Rats with GPEE	10	10	43,6±6,6	0	<0,01
Of left half					
Healthy rats	10	9	3,5±0,8	16,5±4,2	<0,01
Rats with GPPE	10	8	3,7±1,4	5,6±2,3	—

TABLE 3. Concentration of SP (in %) in Left and Right Halves of Lumbar Enlargement of Spinal Cord of Healthy Rats and after Creation of CPEE in Ventral Part of Gray Matter on the Right Side (M ± m)

Animals	Half of spinal cord	
	left	right
Healthy with GPEE on the right	100 (21) 319,2±38* (9)	112,4±11,3 (22) 568,4±159** (9)

Legend. SP concentration measured in picograms per milligram protein and expressed as percentage of arithmetic mean (n = 21) SP concentration in left half of spinal cord of healthy animals. *p_t < 0.05, **p_t < 0.01 compared with control on the same side. Number of animals given in parentheses.

It can be concluded from the results of this investigation that LF are peptides. They are sensitive to the action of proteolytic enzymes but they are not proteins (including TT), for the method of extraction ruled out the presence of high-molecular-weight compounds in the extracts. Inhibition of the effects of LF by naloxone indicates participation of the endogenous opioid system in the mechanism of action of LF on the spinal cord.

The results of this investigation explain why asymmetrical effects of extracts of the whole spinal cord appear under pathological conditions. Numerically, peptide factors of each side affect mainly muscle tone of the ipsilateral hind limb [5]. On the creation of a GPEE in one half of the spinal cord, activity of the peptide muscle tone regulators on that side increases, but on the opposite side it decreases (Table 2); for that reason, effects of the more active side begin to predominate in the action of extracts of the whole spinal cord. It can be postulated that lateralization of the peptide ligands corresponds to asymmetry of distribution of their receptors. It is lateralization of receptors that is responsible for the effect of LF being realized on its "own" side on injection of LF into the recipients.

The increase in the SP concentration in the spinal cord of rats with a GPEE may be the result of hyperactivity of SP-ergic neurons, inducing tonic excitation of both ipsilateral and contralateral motoneurons. We know that during the direct action of SP on the spinal cord it potentiates both ipsilateral and crossed tonic reflexes [13].

Considering the data given above on an increase in SP concentration in the spinal cord during hyperactivity of the propriospinal neuron pool of the ventral horn, it must be pointed out that in a chronic pain syndrome the SP concentration in the ventral horn of the animals' spinal cord is reduced [11].

Creation of a right-sided GPEE causes the same changes in activity of the extracts (Table 1) as a right-sided strengthening of vestibulospinal influences on the spinal cord [5]. The explanation of this may be that vestibulospinal fibers end on interneurons in Rexed's layers VII and VIII, where propriospinal neurons creating the GPEE are evidently located [6]. It is also known that interneurons transmitting excitation to extensor motoneurons during the crossed extensor reflex are the same promotor neurons that are excited by stimulation of the lateral vestibular nucleus of Deiters [9].

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CARDIODEPRESSOR ACTION OF BLOOD SERUM FROM PATIENTS WITH SEVERE SUPPURATIVE INFECTIONS

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UDC 616.94-022.7-036.17-06:
616.127-008.6-02:616.157

KEY WORDS: suppurative infection; isometric contractions; intracellular potentials.

Surgical sepsis is accompanied as a rule by disturbances of myocardial contractility, with clinical manifestations of septic myocarditis [5, 6]. Previous investigations [4] showed the presence of toxic myocardial damage in 83% of patients with surgical sepsis, which developed in association with acute suppurative infection or with extensive post-traumatic purulent wounds. In patients with sepsis the infectious process sometimes develops so rapidly that it leads to toxic (septic) shock, a key factor in which is the direct toxic action on the heart [7]. The blood plasma of animals with experimental septic shock has been shown to depress contractility of the intact myocardium significantly [9]. Data in the literature on the study of the central hemodynamics of patients with sepsis are very contradictory, and both a decrease and an increase in their cardiac output have been reported [1, 8, 11].

The aim of this investigation was to study the direct action of blood serum from patients with severe suppurative infection (septicemia, febrile respective pyemia) on contractility and intracellular potentials of isolated fragments of guinea pig myocardium.

EXPERIMENTAL METHOD

Isometric contractions and intracellular transmembrane potentials in response to electrical stimulation of the isolated auricles of guinea pig atria were studied in three series of experiments (Table 1). In series I each myocardial preparation was perfused with Tyrode solution, during stimulation at constant frequency, followed by replacement of the solution by blood serum from healthy donors or from patients with sepsis. In the experiments of series II, the action of serum from healthy blood donors and patients on the same myocardial preparation was compared. In the experiments of series III the frequency-force relationship was investigated during a change in the frequencies of stimulation: 0.1, 0.2, 0.5, 1, and 2 Hz.

Blood from patients with sepsis was taken immediately after admission to the clinic and before treatment had been instituted. The serum used for perfusion in all series of experiments was diluted with Tyrode solution in the ratio of 1:1, oxygenated with carbogen (95% O_2 + 5% CO_2) for 15 min, and the pH stabilized at 7.2-7.25. Next, with the aid of ion-selective electrodes (Radiometer, Denmark) activity of free Ca^{2+} ions was measured, and in the event of disparity, PCA was adjusted in the donors' and patients' sera to the level in Tyrode solution (2 mM). To prevent the serum from frothing during oxygenation, antifoam was used.

Biophysical Research Laboratory and Laboratory of Clinical Physiology, Department of Wounds and Wound Infection, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 12, pp. 660-663, December, 1987. Original article submitted March 26, 1987.