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POSTURAL CHANGES IN HEALTHY RATS AFTER INTRACRANIAL INJECTION OF BRAIN EXTRACTS FROM ANIMALS WITH EXPERIMENTAL VESTIBULOPATHY

G. N. Kryzhanovskii, V. K. Lutsenko, M. Yu. Karganov, and V. I. Torshin

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It has been suggested that brain activity under normal and pathological conditions is accompanied by the production of substances capable of inducing similar states in animals not exposed to that particular experimental situation [2-4, 9]. After injury to the cerebellum, substances acting on the spinal cord of healthy animals in the same way as suprasegmental structures in this form of pathology have been discovered in brain extracts [2, 3, 7]. In the present investigation the possibility of reproducing a pathological state in healthy recipients by means of extracts of injured brain was studied on a model of experimental vestibulopathy [5]. A special feature of the investigations was that unilateral injury to the vestibular system was confined to Deiters' nucleus, and in some cases hyperactivation was induced, whereas in others the nucleus was destroyed, and these procedures were reflected in clinically different types of vestibulopathy. An evident advantage of this experimental model, and one that is very important for evaluation of the effects, is that the vestibular rotation syndrome is readily observable.*

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. Prolonged hyperactivity of the lateral vestibular nucleus (LVN) was induced with the aid of tetanus toxin (microinjection of 2 MLD in a volume of 0.04 µl). LVN was destroyed by electrocoagulation (current 10 mA, duration 15 sec). On the side of destruction of LVN the rats' neck and trunk were flexed and the limbs were flexed and abducted. On the other side the two limbs were extended. In rats with hyperactivity in LVN no changes of posture were present at rest. All animals developed rotation relative to the long axis of the body toward the nucleus with lower activity, i.e., to-

*The preliminary results of this investigation were published previously [4, 6].

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TABLE 1. Changes in Muscle Tone of Recipients after Injection of Brain Extracts from Rats with Vestibulopathy

Experimental conditions	Substances injected into recipient	Total number of animals	Delay in drawing in hind limb (n)			Arching of the spine (n)	Hypertonia of the tail (n)	P_{cs}	Duration of delay in drawing in hind limb, sec		P_t
			left	right	both				right	left	
Activation of right-sided LVN of donor											
LMWC after destruction of left LVN; EG in right LVN		16	0	13	—	—	—	<0,05	—	—	—
	LMWC	8	0	8	—	2	1	<0,01	23,8±5,6	0	<0,001
	Active fraction 1	14	0	14	4	8	1	<0,01	22,5±3,9	0	<0,001
	Active fraction 2	7	0	7	0	4	0	<0,01	—	—	—
	Other fractions	12	0	0	3	0	2	—	—	—	—
	LMWC after proteolysis	10	1	1	0	7	1	—	0,8±0,8	0,5±0,5	—
	Nalorphine + LMWC	10	1	2	0	4	2	—	2,7±1,8	1,0±0,3	—
	Naloxone + LMWC	8	0	0	1	4	0	—	0	0	—
Activation of left-sided LVN of donor											
LMWC after destruction of right LVN; EG in left LVN		16	13	1	—	—	—	<0,05	—	—	—
	LMWC	15	14	0	—	10	5	<0,01	0	30,3±6,8	<0,001
	Active fraction 1	3	3	0	1	1	0	—	—	—	—
	Active fraction 2	13	12	0	1	9	0	<0,01	0,8±0,8	25,8±5,6	<0,001
	Other fractions	9	1	0	1	3	3	—	—	—	—
	LMWC after proteolysis	10	0	1	0	5	0	—	1,0±1,0	0	—
	Nalorphine + LMWC	10	1	1	0	7	2	—	2,5±0,8	1,2±0,4	—
	Naloxone + LMWC	8	1	0	0	2	1	—	0	1,2±1,2	—

Legend. n) Number of recipients in which a given symptom was found, 0) no effect, P_{CS}) significance of predominance of effect on left or right side estimated by criterion of signs, P_t) significance of differences in duration of delay of drawing in (by Student's t test).

ward the side of the destroyed LVN or to the side opposite to the generator of pathologically enhanced excitation (EG) in LVN [5].

One day later the brain of the rats with vestibulopathy was removed to extract low-molecular-weight components by the method in [7]. The extract was neutralized and the residue separated and lyophilized. The substances thus obtained were fractionated on a column packed with Sephadex G-15 (elution with 0.06 M $NaHCO_3$ solution). The biologically active fraction of low-molecular-weight components (LMWC) eluted in the actinomycin D zone (1255.5 daltons) was chromatographed on a column with finely dispersed Sephadex G-50 (50 mg was applied in 1.0 ml water, column 1.5 × 35 cm, elution with 0.06 M $NaHCO_3$ solution). Samples with significant absorption at 206 nm were subjected to biological tests. One of the fractions (this will be referred to later as "active fraction 1"), giving rise to an asymmetrical response, was chromatographed on a column packed with Sephadex G-50 (12 mg was applied in 0.5 ml water, column 1 × 15 cm, elution with water). The fraction of eluate from this column causing postural asymmetry will be described below as "active fraction 2."

To study sensitivity to proteolysis, the LMWC (6 mg of the dry substance in 1 ml) were incubated with pronase (0.1 mg/ml, specific activity 45,000 units/g, from Calibochem, USA) for 2 h at 37°C. The reaction was stopped by boiling in a water bath for 10 min.

Neutralized solutions of LMWC or separate fractions, in a volume of 100 µl (0.35-1.2 mg of the dry substance) were injected slowly into the cisterna magna or lateral ventricle of rats anesthetized superficially with ether. The cannula for injection was implanted into one of the lateral ventricles a few days before the experiment. Nalorphine (from Chinoin, Hungary) was injected intraperitoneally in a dose of 10 mg/kg 5 min before injection of the

test solutions; naloxone (from Endo Laboratories, USA) in a dose of 2 μg was injected into the cisterna magna simultaneously with LMWC (600 μg/0.1 ml).

EXPERIMENTAL RESULTS

From 5 to 15 min after intracranial injection of the solution of LMWC and the active fractions into healthy animals contraction of the trunk muscles and a slight increase in tone of the tail muscles, manifested as delayed dropping of the tail after passive raising, were observed (Table 1). Later characteristic changes took place in the tone of the hind limb muscles. The recipients' hind limb could be displaced backward or to one side on the side where LVN of the donors was more active (on the side of EG or the contralateral side in the case of destruction of LVN). The limb remained in this position for 10-60 sec. On the opposite side the response to displacement of the hind limb was normal — the passively abducted limb was immediately drawn in by the rat. The difference in the time during which the rat held the limb in the assigned position and the occurrence of the effects predominantly on one side were significant (Table 1). These changes in muscle tone disappeared 30-40 min after injection of the extracts.

The investigations thus show that responses of healthy animals to injection of the extracts corresponded to the type of vestibulopathy and the side of injury to LVN in the donors.

Preliminary data were obtained on the chemical nature of the active factors tested. The method of extraction of the LMWC [7] and also the low values of extinction of the active fractions at wavelengths of over 250 nm makes the suggestion that they contain nucleic acids and proteins unlikely. The low molecular weight (the active substances were eluted from the column at the same time as reference substances with a molecular weight of about 1000 daltons), their sensitivity to proteolysis (Table 1), and their thermostability (withstanding boiling for 5 min) point to the peptide nature of the factors studied, and the abolition of their action on the brain by competitors of opiates such as nalorphine and naloxone is evidence that opiate systems play a role in the formation of the reactions evoked in the recipients.

Postural asymmetry evoked by the extracts in the recipients is a specific response. Despite the similar procedure of introduction of electrodes or the cannula into the donors LVN and the similar technique of extraction of the active factors from the brain, a clear difference was obtained in the character of the asymmetry induced in the recipients according to whether LVN was destroyed or stimulated (Table 1). The character of the asymmetry did not depend on the site of injection (right or left lateral ventricle, cisterna magna). Injection of the substances into the animals in the same concentration and volume, but after proteolysis or in combination with opiate antagonists, did not evoke asymmetrical responses in the recipients (Table 1). Fractions eluted from the column earlier or later than the active fraction likewise did not evoke asymmetrical responses, even if they were injected in higher concentrations.

The results are evidence that substances isolated from the brain of rats with different forms of vestibulopathy evoke changes in tone of the hind limb muscles of healthy animals similar to those present in the donor. Meanwhile, rolling over of the recipient rats was not observed in any of the experiments. This was perhaps due to several circumstances: the insufficient quantity of active factor in the LMWC of the extracts, the increased tone of the neck and trunk muscles of the recipients, hindering labyrinthine and neck reflexes, the limitation of the animals' mobility, and preservation of some corrective influences that were abolished in the donors by the pathological process. The problem of the specific association of the test factors with the vestibulopathy is a matter for further investigation. In connection with the data showing the possible role of opiate systems in the formation of asymmetry in the recipients it must be pointed out that synthetic enkephalins can induce asymmetry of muscle tone in spinal rats [1]. It may be that unilateral activation of the vestibular system brings to light substances whose distribution and reception in the nervous system are asymmetrical.

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STATE OF THE SARCOLEMMA OF SUBENDOCARDIAL PURKINJE CELLS IN THE LATE STAGE OF EXPERIMENTAL MYOCARDIAL INFRACTION IN DOGS

L. V. Rozenshtraukh, V. G. Sharov, and A. M. Vikhert*

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KEY WORDS: Purkinje cells; sarcolemma; myocardial infarction.

Morphological studies have shown that occlusion of the left descending coronary artery in dogs gives rise to necrosis in the myocardium of the left ventricle; cell death takes place primarily in the subendocardial region of the myocardium, after which the process of injury spreads toward the epicardium. Between 6 and 24 h after the beginning of ischemia, the infarct as a rule becomes transmural, i.e., most of the myocytes in the zone of ischemia have suffered irreversible injury [12]. In this late stage of experimental infarction persistent disturbances of rhythm develop, due to the activity of subendocardial Purkinje fibers located in the zone of ischemia [5, 9, 14]. Despite the fact that after occlusion of the coronary artery the earliest and most profound changes arise in the region of the endocardium, the subendocardial elements of the conducting system preserve their viability and act as sources of arrhythmias in the late stage of experimental myocardial infarction.

The subendocardial Purkinje fibers isolated from the zone of ischemia in the late stage of infarction are known not to have undergone any significant changes [6].

The object of this investigation was to study the ultrastructural features of the subendocardial Purkinje fibers in the zone of ischemia, paying special attention to the state of the sarcolemma, changes in the structure of which may be responsible both for the development of arrhythmias and for the high sensitivity of the Purkinje cells to antiarrhythmic drugs in the late stage of experimental myocardial infarction [4].

EXPERIMENTAL METHOD

Experiments were carried out on three mongrel dogs of both sexes weighing 10-15 kg. Under pentobarbital anesthia (35 mg/kg, intravenously) a myocardial infarct was induced by two-stage occlusion of the left descending coronary artery [7]. The dogs were reanesthetized 24 h after ligation of the coronary artery (100 mg/kg chloralose, intravenously) and the chest opened; the heart was quickly removed, the left ventricle was cut out and placed epicardium uppermost in oxygenated Tyrode's solution containing (in mM): NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.6, $NaHCO_3$ 20, glucose 5, pH 7.4; the temperature of the solution was 20°C. Material for electron-microscopic investigation was taken from the most

*Corresponding Member of the Academy of Medical Sciences of the USSR.

Laboratory of Electrophysiology of the Heart and Department of Human Cardiovascular Pathology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 10, pp. 406-410, October, 1981. Original article submitted June 20, 1980.

