EFFECT OF VERAPAMIL ON DEVELOPMENT OF THE PAIN SYNDROME IN RATS AFTER SCIATIC NERVE DIVISION

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UDC 616.833.58-089.85-06:
616.8-009.7/-02:615.
217.24/-092.9

KEY WORDS: pain syndrome; verapamil; electrical activity of the rat brain; microcirculation

Division of sensory nerves or of dorsal roots of the spinal cord causes a pain syndrome in animals [3, 7, 14], due to hyperactivation of nociceptive neurons in the dorsal horns of the corresponding segments of the spinal cord [5, 10], which form a generator of pathologically enhanced excitation (GPEE) [1]. An essential role in hyperactivation of neurons in response to nociceptive stimuli is ascribed to increased release of excitatory amino acids in the dorsal segments of the spinal cord [13], the mechanism of whose action on target cells is determined by an increased inflow of Ca^{2+} ions [4, 16]. It was shown previously that application of verapamil, a calcium channel blocker, to the dorsal surface of the spinal cord in the region of the GPEE induces suppression of a pain syndrome of spinal origin [2].

The aim of this investigation was to study the effect of verapamil on the development of a pain syndrome in rats after division of the sciatic nerve and in response to systemic administration of the drug.

EXPERIMENTAL METHOD

Experiments were carried out on 32 male Wistar rats weighing 180-200 g at the beginning of the investigation. To create a pain syndrome in the rats, the sciatic nerve was divided under hexobarbital anesthesia (15 mg/kg, intraperitoneally) at the level of the popliteal fossa, after which the central end of the divided sciatic nerve was placed in a polyethylene capsule and left in situ in the wound. The denervated animals were divided into two groups. The first group comprised rats receiving verapamil with drinking water perorally, daily after division of the nerve for 22 days, starting with the 1st day after the operation, and in a daily dose of 5 mg/kg. Rats of the 2nd, control group received water without verapamil. Rats of the 3rd group underwent a mock operation (without division of the sciatic nerve). Development of the pain syndrome in the rats was assessed by measuring the change in thresholds of the nociceptive response, by the appearance of autotomy and also according to the results of recording evoked potentials (EP) in the somatosensory cortex and disturbance of the microcirculation in the mesentery. Thresholds of nociceptive responses in these animals were determined by the hotplate (surface temperature of plate 55°C) test, reflected in the response of paw licking. Evoked potentials in response to electrical stimulation of the tissues of both hind limbs and both forelimbs were recorded by a monopolar technique in the somatosensory cortex of both hemispheres, at their foci of maximal activity, with silver surface electrodes, with a tip 0.8 mm in diameter. The reference electrode was fixed in the region of the frontal sinus. Electrical stimulation of the limbs was applied through bipolar needle electrodes, introduced beneath the skin, with pulses of current 0.1 msec in duration. EP were averaged and

Laboratory of Pathophysiology of Pain and Laboratory of General Pathology of the Microcirculation, Research Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 9, pp. 229-231, September, 1992. Original article submitted July 2, 1992.

0007-4888/92/0009-1217$12.50

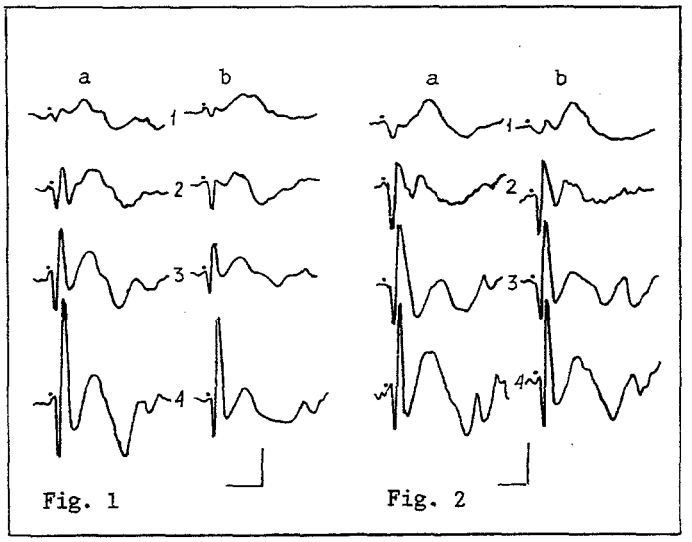

Fig. 1. EP in contralateral somatosensory regions of cerebral cortex in rats not receiving verapamil, during electrical stimulation of tissues of corresponding hind limbs: a) EP during electrical stimulation of tissues of limb with divided nerve; b) EP during electrical stimulation of tissues of intact (opposite) limb, 1, 2, 3, 4) Intensity of stimulation 1, 2, 3, and 4 times the threshold respectively. Calibration: 1 mV, 100 msec.

Fig. 2. EP in contralateral somatosensory regions of cerebral cortex in rats receiving verapamil, during electrical stimulation of tissues of corresponding hind limbs: a) EP during electrical stimulation of tissues of limb with divided nerve; b) EP during electrical stimulation of tissues of intact (opposite) limb. 1, 2, 3, 4) The same as in Fig. 1.

assessed on the basis of 10 presentations by a specialized computer. Recording of EP began 25 days after sciatic nerve division. For this purpose the animals were anesthetized with ether and fixed in a stereotaxic apparatus, artificially ventilated, and immobilized with suxamethonium. The microcirculation was studied in the mesentery 25 days after sciatic nerve division in rats anesthetized with pentobarbital (5 mg/100 g), by a method described by the writers previously [2, 3]. The significance of differences between the results was assessed by Student's t test.

EXPERIMENTAL RESULTS

In rats not receiving verapamil, division of the sciatic nerve led after 20-25 days to a significant lowering of thresholds of the pain response to thermal nociceptive stimulation down to 62.3 ± 3.2% of the preoperative level. In animals receiving verapamil, lowering of the pain thresholds was not observed. Meanwhile differences also were found in the animals' behavior. Rats not receiving verapamil became restless and licked and bit the operation wound vigorously, leading to the appearance of signs of autotomy. By the 21st day after the operation, manifestations of autotomy were found in all the animals of this group. Autotomy was not observed, however, in the group of animals receiving verapamil.

TABLE 1. Symptoms of Microhemocirculatory Disturbances in a Deafferentation Pain Syndrome Induced by Sciatic Nerve Division, and during Its Prevention by Verapamil

Experimental conditions	Severity of microhemocirculatory disturbances, number of cases in % of number of animals in series						
	slowing of blood flow in venules	aggravation of erythrocytes: in capillaries	aggravation of erythrocytes: in venules	plasmatic vessels	extravasates of erythrocytes in venules	stasis	pavementing of leukocytes in venules
I Mock operation (n = 8)	0	5	0	0	0	0	20
II Division of sciatic nerve (n = 5)	80*	60*	20	20	0	0	100*
III Division of sciatic nerve + varapamil (n = 7)	14**	14	0	0	0	0	42**

Legend. Here and in Table 2, n) number of animals, *) difference significant between series I and II ($p < 0.05$), **) difference significant between series II and III ($p < 0.05$).

TABLE 2. Permeability of Venules of Rat Mesentery for Colloidal Carbon Particles in Deafferentation Pain Syndrome Evoked by Sciatic Nerve Division, and during Action of Verapamil

Experimental conditions	Extent of disturbances of permeability: number of meserteric windows with and without label, % of total number examined			Intensity of disturbances of permeability: number of rats with different degrees of labeling, % of total number of rats in series				
	without label	1-10 labeled vessels	over 10 labeled vessels	0	I	II	III	IV
I Mock operation (n = 8)	97	3	0	88	12	12	0	0
II Division of sciatic nerve (n = 5)	84*	14	2	20*	80*	80*	60*	20*
III Division of sciatic nerve + verapamil (n = 7)	97**	3	0	57	42	14**	0**	0**

Recording cortical electrical activity of the rats also revealed significant differences between groups of animals receiving and not receiving verapamil. In rats not receiving verapamil, 21 days after division of the sciatic nerve hypersynchronized discharges and paroxysmal activity, in the form of pointed waves in the hemisphere contralateral to the divided nerve were recorded on the electrocorticogram. During electrical stimulation of the tissues of the limb with the divided nerve the amplitude of EP in the contralateral hemisphere was increased compared with the amplitude of EP arising in the contralateral hemisphere in response to stimulation of the symmetrical point of the intact limb (Fig. 1). The threshold of appearance of EP during stimulation of the limb with the divided nerve was lower than that during stimulation of the intact limb, and amounted to 0.71 ± 0.1 and 1.2 ± 0.17 mA respectively. These changes may be the result of stronger stimulation from the GPEE formed in the dorsal horns of the spinal cord after deafferentation (division of the sciatic nerve). In animals with a divided sciatic nerve considerable expansion of the focus of maximal activity (FMA) likewise was observed: the area of FMA in the hemisphere contralateral relative to the divided nerve was more than 4 times greater than that of the contralateral EP during stimulation of the right (intact) limb. The results are evidence of increased excitability and synchronization of neuronal activity in the cortex of the hemisphere contralateral relative to the divided nerve. In animals receiving verapamil no difference was found between the thresholds of appearance or the amplitude of EP in response to electrical stimulation of tissues of the intact and denervated hind limbs (Fig. 2). Moreover, in rats receiving verapamil, the threshold of onset of the ipsilateral EP in response to stimulation of the tissues of the denervated and intact hind limbs was equal, whereas in rats not receiving verapamil, and with a developed pain syndrome, the threshold of onset of the ipsilateral EP in response to stimulation of the tissues of the denervated limbs was more than 3 times higher than the threshold of onset of ipsilateral responses during stimulation of the tissues of the intact, symmetrical limb.

The study of the microhemocirculation in the mesentery of rats with a pain syndrome revealed slowing of the blood flow in the venules, increased aggregation of erythrocytes in the capillaries, increased pavementing of the leukocytes in the venules, and changes in venular permeability of the mesenteric microvessels, expressed as an increase in the number of "labeled" microvessels and an increase in the intensity of labeling (Tables 1 and 2). In addition, an increase in the number of degranulated mast cells by half, involution of the thymus by 36%, and an increase in the mass of adrenals by 15% were observed in these animals compared with rats of the 3rd group. The disturbances of venular permeability and of the terminal blood flow thus revealed are evidence of a pain syndrome in these animals. In rats receiving verapamil, the above-mentioned parameters of the microhemocirculation and of venular permeability did not differ from those in rats undergoing the mock operation (Tables 1 and 2). No changes were found in the adrenals and spleen.

Thus the results of this study of evoked brain potentials, of behavioral responses, and of the microhemocirculation are evidence that gradual systemic administration of verapamil in animals prevents the development of a pain syndrome after sciatic nerve division. The therapeutic action of verapamil is due to blockade of the increased inflow of Ca^{2+} ions into the cell, which takes place during activation of neurons by nociceptive stimuli [11, 15]. This view is confirmed by data on prevention of development of the pain syndrome in rats after sciatic nerve damage by NMDA-receptor blockers [12], activation of which is known to increase the calcium inflow [4, 6]. It was shown previously that application of verapamil, like that of Mg^{2+} ions, in the dorsal horns of the spinal cord on the region of a GPEE, which induces a pain syndrome, prevents or considerably weakens the latter [2]. It can also be considered that on systemic injection verapamil prevents the development of the GPEE and inhibits its activity in the dorsal horns of the spinal cord after their deafferentation. Another possibility is that verapamil inhibits hyperactivity of other structures of the nociceptive system also and, in particular, the thalamus and cerebral cortex, which are subject to influences from the GPEE, and thus prevents the formation of a pathological nociceptive system, which lies at the basis of the deafferentation pain syndrome. Yet another mechanism may take part in these processes, namely activation by verapamil of the endogenous opioid system, which exerts antinociceptive control, for Ca-channel blockers potentiate the analgesic effects of morphine and stress [8, 9].

The results of the present and previous investigations raise the question of the use of verapamil in combined pathogenetic treatment of the corresponding pain syndromes.

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