level. The sharply increased noradrenalin concentration in l.c. + n.sc. suggests maintenance of a high level of CA synthesis in the noradrenalin-synthesizing nuclei. Noradrenalin is released in s.n. from endings of incoming axons of noradrenalin-synthesizing neurons. The data in Figs. 1 and 2 indicate a different time course of the noradrenalin level in l.c. + n.sc. and s.n., in agreement with data in the literature showing definite differences in CA secretion in bodies of neurons and terminal ramifications of their axons [9].

For 30 days after the end of IM significant changes thus take place in dopa and CA levels in the noradrenalin-synthesizing nuclei of the brain stem (l.c. + n.sc.) and the mesencephalic dopamine-synthesizing nucleus (s.n.). The most marked changes affect the noradrenalin concentration in l.c. + n.sc.: By the 30th day it still remains almost 1.5 times higher than in the control. Survival of the animals after long-term IM suggests that the raised level of noradrenalin in the noradrenalin-synthesizing nuclei of the brain stem under these experimental conditions is protective in character.

LITERATURE CITED

1. T. I. Belova and R. Kvetnanskii (Kvetnansky), Usp. Fiziol. Nauk, 12, 67 (1981).
2. K. V. Sudakov, Emotional Stress and Arterial Hypertension [in Russian], Moscow (1976).
3. R. Kvetnansky and L. Mikulaj, Endocrinology, 87, 738 (1970).
4. R. Kvetnansky, A. Mitro, M. Palkovits, et al., Catecholamines and Stress, ed. by E. Usdin, R. Kvetnansky, and J. Kopin, New York (1976), p. 39.
5. R. Kvetnansky, M. Palkovits, A. Mitro, et al., Neuroendrocrinology, 23, 257 (1977).
6. R. Kvetnansky, Catecholamines and Stress. Recent Advances, ed. by E. Usdin, R. Kvetnansky, and J. Kopin, New York (1980), p. 7.
7. M. Palkovits, Brain Res., 59, 443 (1973).
8. M. Palkovits, R. M. Kobayashi, J. S. Kizer, et al., Neuroendocrinology, 18, 144 (1975).
9. M. Palkovits, E. Mezoy, and A. Feminger, Catecholamines and Stress. Recent Advances, ed. by E. Usdin, R. Kvetnansky, and J. Kopin, New York (1980), p. 21.
10. J. M. Saavedra, R. Kvetnansky, and J. Kopin, Brain Res., 160, 271 (1979).
11. J. M. Saavedra, Brain Res., 160, 37 (1979).
12. A. M. Sauter, Y. Baba, E. A. Stone, and M. Goldstein, Brain Res., 144, 415 (1978).
13. A. Simonyi, B. Kanyicska, A. Feminger, et al., Biogenic Amines, 1, 123 (1984).

PEPTIDERGIC ASYMMETRY OF THE RAT SPINAL CORD

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Neurochemical studies of the brain have shown that dominance of one of the paired structures may be due to pre-existing or acquired asymmetry of distribution of endogenous chemical regulators and, in particular, of mediators and their receptors [8, 9]. There are hardly any data on the neurochemical lateralization of the spinal cord. Yet such data are extremely important for an understanding of the mechanisms of asymmetrical responses of the spinal cord to the action of chemical compounds [1-7].

The aim of this investigation was to study the effect of extracts of halves of the spinal cord (the lumbosacral enlargement) on muscle tone in the hind limbs of rats, changes in the effect of extracts of the whole lumbar division after selective activation of neurons in its right half, and the chemical nature of lateralization factors (LF), which are substances causing asymmetrical changes of muscle tone in the hind limbs.

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TABLE 1. Passive Extension of Hind Limbs of Rats after Injection of Extracts of Lumbar Enlargement of Spinal Cord (M ± m)

Extract of lumbar enlargement	Number of rats		Duration of passive extension of hind limb, sec	
	total	with passive extension	right	left
Right half	10	10*	25,1±5,1	2,6±2,2**
Left half	8	7*	1,1±1,1	25,1±8,2**
Whole enlargement	10	7*	9,9±1,2	1,8±0,9**
Right and left halves	8	7*	5,1±0,8	2,0±0,5**
Whole enlargement, with vestibulopathy	10	10*	22,3±4,1	0
Right half after proteolysis	10	1	0,5±0,5	0
Left half after proteolysis	10	2	0,9±0,4	0
Right half + naloxone	10	0	0	0
Left half + naloxone	10	1	0	1,2±1,2

Note. Asymmetry in distribution of muscle tone estimated 30 min after injection of extract. *$P_{st} < 0.05$, **$P_t < 0.05$.

EXPERIMENTAL METHOD

Non-inbred male rats weighting 250-280 g were used. The animals were decapitated, the lumbrosacral enlargement of the spinal cord was removed, and its tissues were frozen and kept at −18°C. Each frozen fragment of spinal cord was divided into left and right halves under a magnifying glass (magnification 2), the razor blade being oriented along the central sulci on the ventral and dorsal surfaces and the midline of the "butterfly" of gray matter. Material from tissues of the left or right halves of the spinal cord was placed in a glass homogenizer, containing 1 M acetic acid, warmed to 90°C (15 ml of acid to 1 g of tissue). The homogenizer was kept for 5 min in a boiling water bath, after which the tissue was dispersed by means of a glass pestle for 10 min. The tissue suspension was cooled and centrifuged at 8,000 rpm for 15 min in an OPN-8 centrifuge. The supernatant was withdrawn, and its pH adjusted to 7.0 with concentrated ammonia solution. The residue was separated by recentrifugation and the supernatant was lyophilized.

A vestibulopathy was produced in some of the animals by electrolytic destruction of the left nucleus of Deiters, as described previously [6]. In this case the lumbosacral portion of the spinal cord was extracted whole, without separation into right and left halves. The freeze-dried material was dissolved in distilled water and injected into the cisterna magna of intact rats (5 µg of "protein" estimated by Lowry's method [12] in 10 µl of solution) or with the aid of a polyethylene catheter it was injected beneath the dura mater of the spinal cord (2.5 µg of "protein" in 5 µl of solution), into rats cordotomized at the level T5-T7, below the level of section. On testing of the extracts in a series of experiments the "blind" method was used. Changes in muscle tone of the hind limb in intact animals were estimated from the length of time (in seconds) during which the limb could be held in an extended position (the "passive extension" test [6, 7]), and in "spinal" rats from the difference (in millimeters) of the position of the passively abducted hind limb [10]. Proteolysis of the active principle of the extracts was carried out by means of pronase (from Calbiochem, USA) by the method in [13]. The significance of the difference between the samples was judged by Student's test (P_t) and by the signs test (P_{st}).

EXPERIMENTAL RESULTS

Intracisternal injection of extracts of each half of the rats' spinal cord into healthy recipient rats caused changes in muscle tone of the homonymous hind limb: The duration of its passive extension was about ten times longer than that of the contralateral limb (Table 1). Calculations show that each half of the healthy rat spinal cord contains LF in an amount sufficient to induce asymmetry of muscle tone of the hind limbs in tens of healthy recipients.

TABLE 2. Asymmetry of Muscle Tone of Hind Limbs of Spinal Rats after Application of Extracts of Lumbar Enlargement to Spinal Cord (M ± m)

Donors	Extract of lumbar enlargement	Predominance of muscle tone of hind limb					P_{st}
		number of animals			difference in position of limbs, mm		
		total	tone higher on the		tone higher on the		
			right	left	right	left	
Healthy rats	Right half	10	8	1	6,1±1,6	—	<0,05
	Left half	9	0	9	—	7,5±1,2	<0,05
Rats with vestibulopathy	Total enlargement	9	4	2	0,9±0,5	—	—
	Total enlargement	10	9	0	5,8±0,5	—	<0,05

Note. Asymmetry of position of limbs estimated 60 min after application of extract to spinal cord. Extensor tone in rats with vestibulopathy shown for right hind limb.

Factors determining whether the effect is on the right or left half of the body are evidently qualitatively different, and lateralization of the responses cannot be explained by opposite effects of different doses. The experiments showed that when the effect of an extract of the right half of the spinal cord is exerted on the same side, this is not changed if the dose usually injected (5 μg of "protein") is reduced tenfold or a hundredfold.

After injection of extracts of the whole lumbar enlargement of the longer duration of passive extension was found on the right, although the effect was much less than that of extract of only the right half of the spinal cord (Table 1). Predominance of the right-sided effect was observed also when a mixture of extracts of the left and right halves of the spinal cord, which equal contents of extracted substances, expressed as "protein," was injected (Table 1).

The results are proof of the asymmetry of LF distribution in the spinal cord; however, the site of action of LF (spinal cord or brain, or both these parts of the CNS?) remained unknown. To study this problem, in the next series of experiments the tissue extracts were applied directly to the dorsal surface of the lumbar region of the spinal cord. It was found that extracts of both left and the right half of the spinal cord increased muscle tome of the homonymous hind limb of "spinal" animals (Table 2). This suggests that the distribution not only of LF themselves, but also of the receptor of these compounds, is lateralized in the spinal cord.

Changes also were studied in the balance of activities of LF on the left and right sides under conditions of right-sided activation of neurons on the right side. After destruction of the left Deiters' nucleus in rats, extensor tone disappeared on the left side and extensor placing of the right hind limb occurred. The spinal cord "remembered" the asymmetry of activity of its neurons: Muscle tone of the right hind limb remained higher also after cordotomy performed 24 h after coagulation of the left Deiters' nucleus (11.0 ± 2.7 mm, n = 6). It follows from Tables 1 and 2 that right-sided activation of the spinal cord by impulses conducted along the vestibulospinal tract leaves its neurochemical "trace." In animals with vestibulopathy, extracts of the lumbar region of the spinal cord evoked right-sided changes in muscle tone of the hind limbs; the effect was more marked, moreover, in the intact and "spinal" recipients than when extracts of the whole lumbar division from healthy rats were used, and it was comparable with that for extracts of the right half of the spinal cord (Tables 1 and 2).

Investigation of the chemical nature of LF of the spinal cord showed that they are inactivated after incubation with pronase (Table 2). However, unlike proteins, the LF are thermostable — they can withstand heating in a boiling water bath. After injection of naloxone, an antagonist of opiate receptors, into the animals, the action of LF was abolished (Table 1). These data are evidence that LF are peptide in nature and that opioid systems are involved in the mechanisms of their action on the CNS.

The results of these investigations are interesting from several points of view. It will be evident that lateralization (neurochemical and functional) is not a unique characteristic confined to the brain. In healthy cats segmental monosynaptic spinal reflexes are significantly higher on the right than on the left side [11]. Predominance of right-sided

effects in the action of extracts of whole spinal cord (Tables 1 and 2) indicates the possible neurochemical basis of asymmetry of segmental reflexes.

The ability of extracts of halves of the brain [2, 5, 7] and spinal cord (Tables 1 and 2) to induce effects confined to the homonymous side of the spinal cord is proof that the distribution of LF in these two parts of the CNS is similar in principle of distribution of LF in the nervous system, reflecting the similarity of peptide composition of brain and spinal cord LF and the identical direction of changes in peptide concentrations in several situations, taken together, explain the hitherto unexplained fact that in vestibulopathy asymmetry of muscle tone of the recipient's hind limbs is caused by extracts not only of the spinal cord (Tables 1 and 2), but also of the brain [6]. Asymmetry of the effects of LF is perhaps linked with asymmetry of opiate receptors, as is shown by abolition of the action of LF in the presence of the opiate antagonist. This hypothesis is in agreement with data on asymmetrical changes of muscle tone under the influence of opioid peptides [1, 4].

LITERATURE CITED

1. G. Ya. Bakalkin, K. N. Yarygin, E. D. Trushina, et al., Dokl. Akad. Nauk SSSR, 252, No. 3, 762 (1980).
2. G. Ya. Bakalkin, K. N. Yarygin, A. G. Kobylyanskii, et al., Dokl. Akad. Nauk SSSR, 260, No. 5, 1271 (1981).
3. G. A. Vartanyan and Yu. V. Balabanov, Byull. Éksp. Biol. Med., No. 8, 147 (1978).
4. G. A. Vartanyan, B. I. Klement'ev, E. I. Varlinskaya, et al., Byull. Éksp. Biol. Med., No. 3, 50 (1982).
5. M. Yu. Karganov, Patol. Fiziol., No. 5, 93 (1981).
6. G. N. Kryzhanovskii, V. K. Kutsenko, M. Yu. Karaganov, et al., Byull. Éksp. Biol. Med., No. 10, 404 (1981).
7. G. N. Kryzhanovskii, V. K. Lutsenko, and M. Yu. Karganov, Byull. Éksp. Biol. Med., No. 1, 14 (1982).
8. V. K. Lutsenko and M. Yu. Karganov, Neirokhimiya, 4, No. 2, 197 (1985).
9. S. D. Glick, D. A. Ross, and L. B. Hough, Brain Res., 234, 53 (1982).
10. C. Giurgea, J. Daliers, and M. L. Rigaux, Arch. Int. Pharmacodyn., 191, 292 (1971).
11. H. Hultborn and J. Malmsten, Acta Physiol. Scand., 119, 423 (1983).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. A. Tsugita and S. Akabori, J. Biochem., 46, 695 (1959).

EFFECT OF VITAMIN E DEFICIENCY ON CARDIAC ARRHYTHMIAS INDUCED BY ACUTE ISCHEMIA

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Recent investigations have shown that activation of lipid peroxidation (LPO) due to stress, acute ischemia [2], and also direct induction of LPO [4] in the isolated atrium lead to injury to cardiomyocyte membranes and may thus play a role in the genesis of cardiac arrhythmia and fibrillation, which are prevented by synthetic antioxidants [2, 4]. In this connection it seemed probably that a deficiency of the principal natural antioxidant and membrane stabilizer, α-tocopherol (TP), may reduce the resistance to the heart to arrhythmias and fibrillation, that frequently arise in response to acute ischemia and which constitute the main cause of sudden death.

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