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ASYMMETRICAL CHANGES IN HIND LIMB MUSCLE TONE IN RATS AFTER INJECTION OF EXTRACTS FROM THE LEFT AND RIGHT HALVES OF THE BRAIN

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Brain extracts from rats with unilateral injuries [2-4] or with stimulation [7] of symmetrical structures, if injected into healthy animals, give rise to postural changes in the latter which depends on the character and side of the intervention of the donors. For instance, intracranial injection of brain extracts from animals with experimental vestibulopathy due to hyperactivation or destruction of the vestibular nuclei of Deiters [8] is accompanied by changes of muscle tone in the recipients, which may be in various directions [7]. The further study of this phenomenon was dictated by the need to explain the more general problem associated with chemical lateralization of the brain [12], namely to study the effects of extracts from the left and right halves of the normal brain.†

EXPERIMENTAL METHOD

Albino rats weighing 150-200 g were used. The animals were decapitated, the brain with the cerebellum and medulla was removed, and the brain was divided along the midline into right and left halves, frozen in liquid nitrogen, and kept at −20°C. The frozen tissue was placed in a homogenizer containing 1M acetic acid warmed to 90°C, the homogenizer was kept for 5 min in a boiling water bath, after which the tissue was dispersed for 10 min with a glass pestle [9]. In some experiments extraction with a mixture of chloroform and methanol was used [11]. The suspension was cooled and centrifuged at 10,000 rpm for 15 min (K-24, East Germany). The supernatant was collected and its pH adjusted to 7.0 with concentrated ammonia solution. The residue was separated by filtration and the resulting solution lyophilized. To study the sensitivity of the extracts to proteolysis, a preparation of pronase was used (specific activity 45,000 units/g, from Calbiochem, USA). Samples 1 ml in volume, containing 1.2-1.6 mg "protein" [13] of the extracts, 100 μg pronase, 5×10^{-4}M $CaCl_2$ in 0.05 M borate buffer, were incubated at 37°C for 2 h. The reaction was stopped by immersing the samples in a boiling water bath and heating them for the next 10 min. In some experiments

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TABLE 1. Passive Extension of Hind Limbs in Rats after Suboccipital Injection of Extracts of Halves of the Brain

Substance injected into recipient	Number of experiments	Delay of adduction of ipsilateral hind limb (number of animals)	P_{CS}	Number of experiments	Duration of delay of adduction of hind limb (M ± m), sec		P_t
					right	left	
ERH	16	16	<0,01	9	28,9±4,7	0	<0,001
ERH after proteolysis	10	1	—	10	0,8±0,8	1,5±1,5	—
ERH + nalorphine	10	2	—	10	1,5±1,1	2,2±0,3	—
ELH	23	21	<0,01	19	0	26,6±2,9	<0,001
ELH after proteolysis	10	2	—	10	0,5±0,5	1,3±0,8	—
ELH + nalorphine	10	1	—	10	0,7±0,7	1,0±1,0	—

Legend. Here and in Tables 2 and 3: ERH) extract of right half of brain, ELH) extract of left half of brain; P_{CS}) reliability of attribution of effect to ipsilateral side, P_t) significance of difference in time of delay of adduction of hind limbs.

TABLE 2. Effect of Naloxone on Effects of Suboccipital Injection of Extracts of Halves of Rat Brain (M±m)

Substance injected into recipient	Number of experiments	Delay of adduction of hind limb, sec	
		right	left
15 min after injection of extract			
ELH*	6	0,8±0,8	21,0±2,5
ERH	6	17,0±4,5	1,0±1,7
ELH	6	0,3±0,5	16,0±8,1
25 min after injection of extract			
ELH + physiological saline	6	0,8±0,5	17,0±1,0
ERH + naloxone	6	2,7±2,0	0,7±0,4
ELH + naloxone	6	0	0

*Control series in which change in action of extracts with time and nonspecific effects connected with repeated injection of solutions (in this case physiological saline) were taken into account.

TABLE 3. Effect of Application of Extracts of Halves of Brain to the Spinal Cord

Substance injected into recipient	Number of experiments	Adduction of ipsilateral hind limb (number of animals)	P_{CS}	Number of experiments	Increase in amplitude of EA, in % of initial level (M ±m)					
					flexors			extensors		
					right	left	P_t	right	left	P_t
ERH*	15	14	<0,01	6	173±182	587±327	>0,1	273±114	83±47	<0,05
ELH	14	13	<0,01	7	27±2	11±10	>0,05	21±21	607±43	<0,001
Physiological saline	10	0	>0,05	—	—	—	—	—	—	—
4-AP	6	1	>0,05	6	10±25	50±50	>0,1	89±84	55±34	>0,1
ERH + 4 = AP	10	8	<0,05	10	79±23	56±34	>0,1	152±35	35±16	<0,01
ELH + 4 = AP	7	6	<0,05	7	112±38	56±44	>0,1	72±48	640±89	<0,001

Legend. P_t) Significance of difference in increase in EA of symmetrical thigh muscles: *Increase in amplitude of EA 30 min after application of solution of extract, in all other cases 1 h after application.

solutions of the freeze-dried preparations (120-160 µg "protein" in 10 or 100 µg water) were slowly (20-30 sec) injected into the cisterna magna of the animals under superficial ether anesthesia. The skin and muscles of the neck were first anesthetized with 0.5% procaine solution. Postural changes, the mobility of the animal, and the response to passive abduction of the limbs were assessed. In the experiments to study the effects of nalorphine (from Chinoin, Hungary), preliminary intraperitoneal injection of 0.5 ml of the drug in a dose of

10 mg/kg was given 5 min before the extracts. Naloxone (from Endo Laboratories, USA; 2 μg/100 μl) was injected intracisternally after intracisternal injection of 120-160 μg "protein" of extracts in 10 μl. Postural changes and the animal's responses were observed 10 min after injection of naloxone. In control experiments the extract and 100 μl of physiological saline were injected.

The action of the extract from the spinal cord was studied in two series of experiments. In one series, just as in [2], the spinal cord was exposed at the level T5-T7, a polyethylene catheter (external diameter 0.5 mm) was introduced through an incision in the dura, and the catheter was manipulated in the caudal direction as far as the lumbar enlargement. Through the catheter 50 μl of a solution of the freeze-dried extract (120-160 μl "protein") was applied directly to the lumbar segments, the catheter was removed, and the spinal cord was ligated in such a way that the intradural space of the caudal part of the spinal cord, which contained the injected substance, was closed. Changes in hind limb muscle tone and responses to pinching were then investigated. In another series of experiments the spinal cord was divided at the level T5-T7 and 100 μl of a solution of extract (unknown to the experimenter; "blind" experiments) was applied to the surface of the cross-section through the caudal parts of the spinal cord. Integral electrical activity (EA) of the extensor (m. quadriceps) and flexor muscles of the knee joints of both hind limbs was derived by four pairs of uninsulated steel needles and recorded on an RM-86 polygraph (from Nihon Kohden, Japan). Tonic spike activity (the averaged amplitudes of spikes over a period of 5 sec) was measured on the electromyogram. The results were expressed as percentages of EA before injection of the solution of extract. The significance of differences was estimated by the criterion of signs (CS) and by Student's t test [5].

EXPERIMENTAL RESULTS

Some 5-10 min after suboccipital injection of the preparations the animals began to flex their spines and their mobility was restricted. After a further 5-10 min clear differences were observed in the response of the hind limbs to passive abduction (Table 1). After injection of extract of the right half of the brain, the right hind limb of the recipients could be abducted and extended posteriorly (sometimes only posteriorly), and the limb stayed in the position in which it had been placed for 25-30 sec. The left hind limb, however, quickly regained its normal position after similar abduction and extension. Injection of extract of the left half of the brain delayed abduction of the left hind limb (Table 1). The abducted limbs were not paretic and muscle tone was preserved. The responses and posture of the animals returned to normal 30-40 min after injection of the extracts.

In the next series of experiments the sensitivity of the extracts and the effect of opiate antagonists, nalorphine and naloxone, on responses to the extract were investigated. Preliminary intraperitoneal injection of nalorphine and treatment with pronase prevented the onset (Table 1), whereas suboccipital injection of naloxone abolished asymmetry of hind limb muscle tone which had developed (Table 2). Application of extracts to the lumbar segments or cross-section of the spinal cord in the thoracic portion caused adduction of the ipsilateral (relative to the half of the brain from which the extract was obtained) hind limb after 15-60 min, and it continued throughout the period of observation (up to 2 days).

The EA of the muscles of both hind limbs increased 1 h after application of extract from the right half of the brain to the cross-section of the spinal cord, the main increase being found in the extensor of the right and flexors of the left knee joints (Table 3). Extract of the left half of the brain caused the opposite effect — an increase in EA of the extensor of the left knee (Table 3).

A nonspecific increase in activity of spinal neurons after intraperitoneal injection of 0.25 mg/kg of the convulsants 4-aminopyridine (4AP) [14] did not lead to the appearance of asymmetry of EA of the muscles (Table 3). The qualitative difference in the effects of the extracts of the left and right halves of the brain on the spinal cord still persisted after preliminary injection of 4AP (Table 3).

With a decrease in the quantity of the substance injected suboccipitally into the rats to 5 μg "protein" in 10 μl a difference was observed in the activity of extracts from the right and left halves of the brain. The duration of passive exchange of the ipsilateral hind limb was 222.0 ± 2.3 sec for extracts of the right, and 41.0 ± 7.8 sec for extracts of the left half of the brain (n = 7 and 5 respectively; $P_t < 0.001$).

The results of these investigations suggest that the active factors present in the left and right halves of the rat brain are different. Their sensitivity to proteolysis suggests the presence of peptide bonds in their molecules, and abolition of the effect by nalorphine and naloxone indicate that these substances probably belong to the class of opioid compounds or that the opiate systems participate in the mechanism of their effect on muscle tone of the hind limbs.

The appearance of asymmetrical effects during the action of extracts directly on the spinal cord is evidence that receptors for these substances are present in the spinal cord and that their distribution is asymmetrical; the longer persistence of the change in muscle tone in spinal animals may perhaps be linked with the blocking of descending regulatory influences.

Opioid peptides are known to induce changes, including asymmetrical changes, in muscle tone in rats [1, 10]. Asymmetrical brain lesions [2-4, 7] probably disturb the normally existing balance between the endogenous opiates, as is brought to light when their biological effects are investigated.

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