MACRO- AND MICROELECTROPHORETIC INVESTIGATION OF CONSTITUTIVE PROTEINS OF BRAIN STRUCTURES

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The protein composition of structures of the rat brain (premotor cortex, area CA3 of the hippocampus, caudate nucleus, frontal area of the cerebellum) was compared by macro- and microdisc electrophoresis. The protein zones were identified by their relative electrophoretic mobility, relative to the mobility of a "marker" protein (soy trypsin inhibitor). Both methods revealed no significant difference in the composition of the brain structures studied. Comparison of the results of electrophoretic fractionation of brain proteins indicates that the micromethod has greater resolving power.

KEY WORDS: brain proteins; microdisc electrophoresis.

Data on the protein composition of different structures of the mammalian brain are few in number and contradictory in nature. Marked heterogeneity of the protein composition of the motor, sensory, and occipital zones of the cerebral cortex has been described in monkeys [10]. Heterogeneity in the composition of soluble and solubilized proteins has been found in structures of the auditory tract in guinea pigs [6]. Quantitative differences have been found during the study of proteins of the brain stem, geniculate body, cerebellum, and cerebral cortex of mice, and it is only in the composition of the olfactory bulbs that a protein not found in other structures has been identified [9]. Only quantitative differences have been found in the protein composition of the thalamus, basal ganglia, brain stem, cerebellum, and cerebral cortex of monkeys and man [5]. No differences have been found in the protein composition of the gray matter of the frontal, parietal, and temporal lobes of the human cerebral hemispheres [1] or in the protein composition of the hippocampus and the visual and auditory areas of the cortex of the rat brain [11].

The electrophoretic investigations listed above thus failed to answer the question whether brain structures are heterogeneous in their protein composition. No information was given in the papers cited of the resolving power and sensitivity of the methods of electrophoresis used; it can therefore be suggested that the contradictory character of the results depended on the unidentical methods and conditions of fractionation and methods of their quantitative detection.

This paper describes a comparative study of the protein composition of brain structures by the method of disc electrophoresis on the micro and macro scale.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 250-300 g. Weighed samples of tissue (1-3 mg) were quickly taken in the cold from the premotor cortex, area CA3 of the hippocampus, the caudate nucleus, and the right and left frontal areas of the cerebellum. A homogenate of four or five weighed microsamples was prepared as described in [7], using glass tubes with a bore of 4 mm as the homogenizer, by the addition of 5 μl/mg of buffer A (0.5% Triton X-100, 0.25 M sucrose, 0.06 M Tris-HCl, pH 7.4, 0.002 M EDTA, 0.00015 M soy trypsin inhibitor). Centrifugation was carried out for 1.5 h at 10,000g and 4°C. The protein concentration in the supernatant was determined as described in [8]. Macrodisc electrophoresis was performed in 7% polyacrylamide gel [3], using glass tubes 10 cm long and with a bore of 0.6 cm. Instead of a concentrating gel, concentrating buffer B (25% sucrose in 0.06 M Tris-HCl, pH 6.7) was used in a volume of 0.1 ml per tube. Between 400 and 500 μg protein was applied to the gel and electrophoresis was carried out for 2.5 h with a current of 2.5 mA to the gel. The gel was stained with 0.5% Amido Black 10B in 7% acetic acid. The

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TABLE 1. Values of Relative Electrophoretic Mobilities of Solubilized Proteins of Brain Microregions Obtained by Macrodisc Electrophoresis

Brain region	Zone											
	1	2c	2	3	4	5	6	7	8	9	10	11
Premotor cortex:												
R_x	1,0	0,84	0,73	0,70	0,62	0,55	0,50	0,47	0,42	0,38	0,34	0,30
E_r	0,00	0,01	0,01	0,01	0,01	0,02	0,01	0,02	0,01	*	*	*
Hippocampus CA3:												
R_x	1,0	0,82	0,72	0,69	0,62	0,55	0,51	0,47	0,41	0,38	0,34	0,29
E_r	0,00	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	*	0,01
Caudate nucleus:												
R_x	1,0	0,83	0,73	0,70	0,62	0,56	0,52	0,47	0,41	0,38	0,32	0,29
E_r	0,00	0,01	0,01	0,01	0,01	0,02	0,02	0,01	0,01	0,01	*	†
Cerebellum:												
R_x	1,0	0,83	0,72	0,69	0,61	0,54	0,49	0,45	0,41	0,38	0,32	0,26
E_r	0,00	0,01	0,01	0,02	0,01	0,02	0,01	0,01	0,01	*	*	*

Legend. Values of E_r (random errors) were calculated for protein zones with CNRA of $p \geq 0.95$; *) protein zone detected with CNRA of $0.95 > p \geq 0.50$, †) with CNRA of $p = 0.20$.

TABLE 2. Values of Relative Electrophoretic Mobilities of Solubilized Proteins of Brain Microregions Obtained by Microdisc Electrophoresis

Brain region	Zone													
	1	2'	2	2"	3	4	5	6	7	8	9	10	11	12
Premotor cortex:														
R_x	1,0	0,80	0,79	0,76	0,68	0,62	0,55	0,52	0,47	0,41	0,38	0,35	0,30	0,23
E_r	0,00	0,02	0,02	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,02	0,02	0,02	*
Hippocampus CA3:														
R_x	1,0	0,81	0,77	0,76	0,68	0,63	0,58	0,51	0,47	0,42	0,36	0,32	0,27	0,23
E_r	0,00	0,03	0,02	0,02	0,02	0,02	0,02	0,02	0,01	0,01	0,02	0,01	0,01	0,02
Caudate nucleus:														
R_x	1,0	0,83	0,79	0,78	0,69	0,63	0,57	0,51	0,45	0,42	0,38	0,34	0,27	0,24
E_r	0,00	0,05	0,02	0,02	0,02	0,01	0,01	0,01	0,01	0,01	0,01	0,01	*	*
Cerebellum:														
R_x	1,00	0,82	0,78	0,76	0,71	0,65	0,56	0,52	0,47	0,41	0,37	0,33	0,29	0,24
E_r	0,00	0,02	0,02	0,02	0,03	0,02	0,01	0,01	0,01	0,01	0,01	0,01	0,01	*

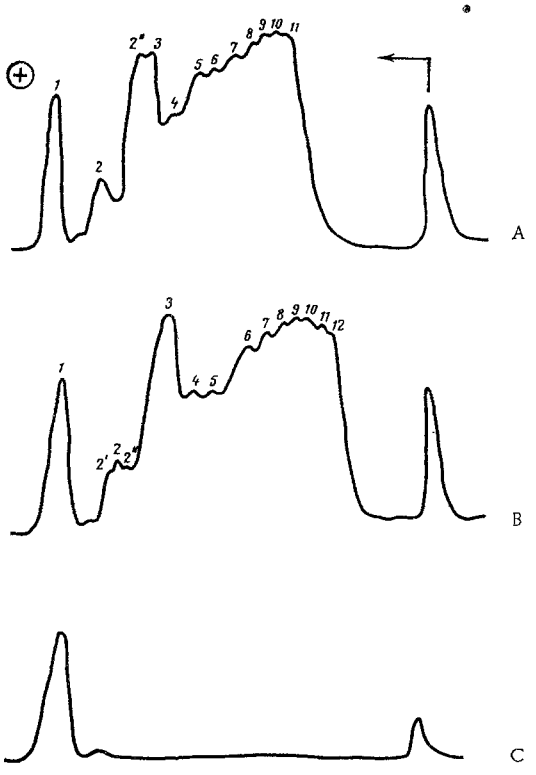

Fig. 1. Densitograms of solubilized proteins of microregions of rat brain obtained by micro- (A) and macrodisc (B) electrophoresis and of "marked" protein (soy trypsin inhibitor – C). Numbers denote protein zones. Arrow marks start and direction of migration of proteins during electrophoresis.

gel with the stained bands was scanned on an "Integraph" densitometer (Switzerland). The sensitivity of this method was about 4 μg protein per zone. The protein composition of each weighed microsample (1-3 mg) of the above-mentioned structures from the right hemisphere and right frontal area of the cerebellum was determined 3 times by microdisc electrophoresis. Capillary tubes with a bore of 0.8-1.0 mm were used as the homogenizer. Homogenization and centrifugation were carried out as indicated above. Microdisc electrophoresis was performed by the method in [4] in capillary tubes 30 mm long with a bore of 0.36-0.40 mm, filled with 7% polyacrylamide gel. Approximately 1 μg protein in a volume of 0.15 μl was applied to the microgel. The microgels were stained with 0.5% Amido Black 10B in 7% acetic acid and the unfixed dye was washed off in 7% acetic acid. The stained gels were scanned in a two-color microphotometer, made by the Design Office of the Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR, in which the sensitivity of the recording system was 5 ng per protein band. The mobility of the protein zones was compared with that of a "marker" protein, namely soy trypsin inhibitor (molecular weight 21,500), the position of which on the gel after electrophoresis coincided with that of the protein zone with the highest electrophoretic mobility.

In all the experiments of this series the frequency of appearance of individual protein zones was determined. The probability of nonrandom appearance of each protein zone was determined by the criterion of signs. When values of the frequencies differed from those given in the tables, the probablity of nonrandom appearance of the protein zone was estimated by the equation [2]:

$$p = 1 - \left[C_n^{m+1} + C_n^{m+2} + \ldots + C_n^n\right](1/2)^n \quad (2)$$

where n is the total number of experiments in the given series; m the number of experiments in which the given protein zone was identified. For protein zones with $p \geq 0.95$ the value of random errors of their relative electrophoretic mobilities was calculated.

EXPERIMENTAL RESULTS

The results of the comparative study of the protein profiles of microareas of the rat brain are given in Fig. 1 and Tables 1 and 2. By macrodisc electrophoresis 9-11 protein zones were distinguished within confidence limits corresponding to a probability of nonrandom appearance (CNRA) of $p = 0.95$; the protein profiles of the paired brain structures, moreover, did not differ from each other. By microdisc electrophoresis 11-14 protein zones were recorded with CNRA of $p = 0.95$. Both methods also revealed a series of protein zones with CNRA of $0.95 > p \geq 0.50$ (Tables 1 and 2). Protein zones 9, of the premotor cortex and frontal area of the cerebellum, 10 in all the structures studied, and 11 of the premotor cortex and frontal area of the cerebellum had CNRA of $0.95 > p > 0.75$ in macroelectrophoresis. It must be emphasized that these same zones were found by microelectrophoresis with CNRA of $p = 0.95$. Protein zone 11, belonging to the caudate nucleus, was recorded with CNRA of $p = 0.20$ by macroelectrophoresis and $p = 0.50$ by microelectrophoresis. Zone 12 was recorded by the micromethod with CNRA of $0.95 > p \geq 0.50$ in the premotor cortex, caudate nucleus, and frontal area of the cerebellum, whereas in the hippocampus this zone was recorded with CNRA of $p = 0.95$; zone 12 was not detected by the micromethod.

The two methods gave approximately equal values of R_X for protein zones starting with the third. The zone with $R_X = 0.79$ in the micromethod probably corresponds to the protein zone with $R_X = 0.84$ in the macromethod, and the difference between their numerical values may be due to destructive changes in the proteins composing this zone as the result of the longer time of electrophoretic fractionation. The same reason could also account for the appearance of protein zone 2° in the macromethod. Protein zones 2' and 2" were not found by macrodisc electrophoresis, evidently because of the diffuse widening of the zones, which is proportional to $\sqrt{t}$, where t is the fractionation time.

The protein profiles of the structures studied were roughly the same and the relative distribution of protein among the zones corresponded to that given in Fig. 1.

It can be concluded from these resuls that there are no significant differences in the protein composition of the structures studied and that the micromethod has the greater resolving power, for both macro- and microelectrophoresis were carried out with approximately the same ratio of amount of protein applied to the gel/sensitivity of the method.

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EFFECT OF THYROTROPIN RELEASING HORMONE AND THYROXINE ON CYTOCHROME OXIDASE ACTIVITY IN THE RAT ADENOHYPOPHYSIS

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Thyrotropin releasing hormone (TRH), in a dose of 0.01 and 1.0 μg/ml, sharply increased cytochrome oxidase activity in the adenohypophysis of rats fed for 6 weeks with methylthiouracil. This effect of TRH on enzyme activity was blocked by thyroxine (T_4), if added to the incubation medium in a concentration of 20 μg/ml. Actinomycin D (20 μg/ml) prevented the blocking of cytochrome oxidase by T_4. TRH in a concentration of 0.01 μg/ml and T_4, in a dose of 2.0 μg/ml, caused no change in cytochrome oxidase activity in the adenohypophysis of intact and partially thyroidectomized rats.

KEY WORDS: thyrotropin releasing hormone; thyroxine; cytochrome oxidase; adenohypophysis.

Little information is available on the effect of releasing hormones on metabolic processes accompanying the secretion of the pituitary tropic hormones. The stimulating effect of thyrotropin releasing horming (TRH) on the formation of $^{14}CO_2$ from [^{14}C]glucose by pig [7, 11] and rat [8] pituitary glands and the blocking of this effect by thyroxine (T_4) have recently been described [7, 11].

Since the intensity of tissue respiration is known to depend on the state of the cytochrome system, including cytochrome oxidase activity [4], it was decided to study cytochrome oxidase activity in rat pituitary glands under the influence of TRH and T_4.

EXPERIMENTAL METHOD

Intact rats and rats in which the sensitivity of the thyrotrophs of the adenohypophysis was increased by partial thyroidectomy or by feeding the animals daily for 6 days (5 mg per animal) with methylthiouracil were used. The adenohypophysis of the rats, after decapitation, was divided into halves (one half was the control, the other experimental). Five or six halves of adenophypophyses were washed, weighed together, and placed in Erlenmeyer flasks with 2 ml of Hanks' medium, aerated with a mixture of 95% O_2 + 5% CO_2 to complete saturation. The samples were then placed in an apparatus of Warburg type for preincubation. After 1 h, TRH (from Hochst, West Germany), T_4, or actinomycin D (from Reomal, Hungary) in 0.5 ml Hanks' medium was added to the experimental samples and 0.5 ml of Hanks' medium only to the control. Cytochrome oxidase activity, de-

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