INCORPORATION OF LEUCINE-^{3}H INTO RAT BRAIN PROTEIN AFTER DESTRUCTION OF VARIOUS NUCLEI SEROTONINERGIC SYSTEMS

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It has been shown that the level of protein and nucleic acid synthesis is an indicator of functional activity of brain structures [2, 6]. The writers showed previously that protein synthesis is under the control of serotoninergic and noradrenergic systems of the brain [5]. In view of differences in the projections of ascending pathways arising from individual nuclei of the serotoninergic system, it was decided to investigate the particular feature of changes in the activity of the brain protein-synthesizing system in animals after selective destruction of these nuclei.

For this purpose the intensity of *de novo* synthesis of high- and low-molecular-weight protein fractions was studied in the motor area of the neocortex, in area CA_3 of the hippocampus, and in the head of the caudate nucleus of rats after destruction, in turn, of the dorsal (NRD) and medial (NRM) raphe nuclei.

EXPERIMENTAL METHOD

Experiments were carried out on 30 male Wistar rats weighing 200-250 g at the moment of operation. NRD was destroyed electrolytically in ten animals and NRM in another ten rats; ten animals undergoing a mock operation served as the control group. Electrolytic destruction of the brain structures was carried out by a stereotaxic method using coordinates taken from Fifkova and Marsala's atlas [9]: for NRD, AP = 6.0, L = 1.5, H = 6.0 mm from the bone surface; the angle of inclination of the electrode in the frontal plane was 14°; for NRM, AP = 6.5, L = 1.5, H = 7.5 mm, angle of inclination of the electrode 12°. The anode was the destructive electrode. The current was 1.5 mA and the duration of coagulation 20 sec. The animals were decapitated 45-50 days after the operation and the brain was removed and dissected at 4°C. Pieces of brain weighing 10-15 mg were taken for biochemical analysis from the right cerebral hemisphere, from the motor area of the neocortex, hippocampal area CA_3, and the head of the caudate nucleus. The choice of brain structures was based on data for specificity of the connections of NRD and NRM with these structures [11]. DL-leucine-2-^{3}H (specific radioactivity 87 mCi/mmole, in a dose of 3 μCi/g body weight) was injected intraperitoneally into the rats 1 h before sacrifice. The tissue was homogenized in three volumes of a solution of 0.06 M Tris-HCl, pH 7.1, containing 0.25 M sucrose and 0.5% Triton X-100. The homogenate was centrifuged for 60 min at 10,000g. Proteins of the supernatant were fractionated on a column with Sephadex G-75 Extra Thin (3 × 140 mm, V_t = 990 μl). Equal volumes of the following fractions were collected from the column: high-molecular-weight proteins (HMWP) with molecular weight of over 70,000, two fractions of low-molecular-weight proteins (LMWP) with molecular weight of 40,000 ± 3,000 and 20,000 ± 3,000 and also an amino acid fraction containing the free labeled precursor. A sample of 0.1 μl was taken from each fraction for microdisk-electrophoresis in 7% polyacrylamide gel (PAG). The remaining volumes of each fraction were transferred to toluene scintillator. The mean values of relative radioactivity, expressed as the ratio of the number of counts per minute for the protein fraction (Y) to the number of counts per minute in the fraction of free amino

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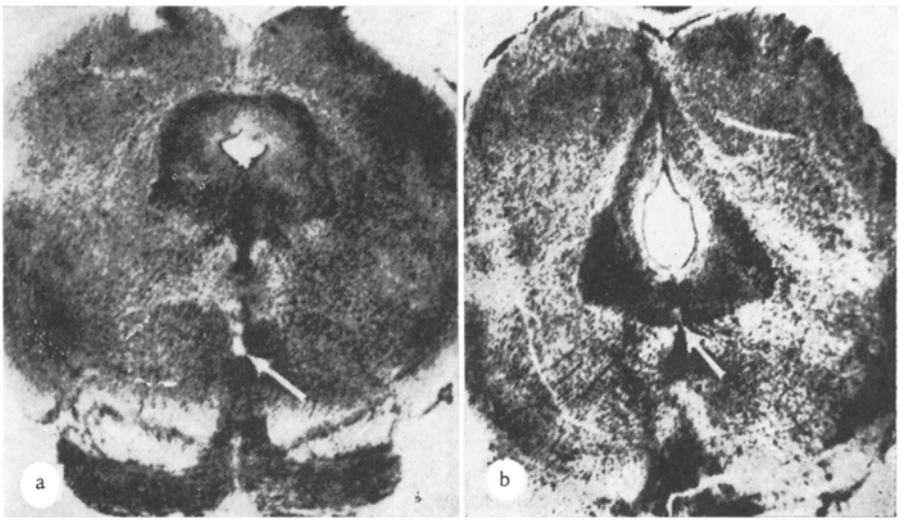

Fig. 1. Localization of destruction in region of medial (a) and dorsal (b) raphe nuclei in the rat brain. Magnification 2.

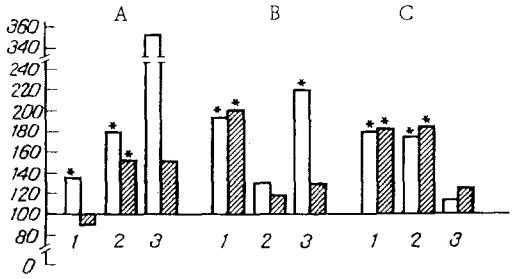

Fig. 2. Changes in incorporation of leucine-^{3}H into HMWP (1) fraction and fraction of protein with mol. wt. of 40,000 ± 3,000 daltons (2) 20,000 ± 3,000 daltons (3) in motor area of neocortex (A), hippocampal area CA_3 (B), and head of caudate nucleus (C) of rats after destruction of medial (unshaded column) and dorsal (shaded column) raphe nucleus. Ordinate, relative radioactivity of protein (in percent of control). Significance of differences at $P < 0.05$ level indicated by asterisk.

acids (X), were calculated by a regression method and expressed as the coefficient of regression $B = \frac{Y}{X}$ [1]. The standard method of comparing regression coefficients [8] was then used. The level of significance for all calculations was 0.05.

To determine the localization and size of the focus of destruction the brain stem was fixed in formalin and embedded in celloidin; sections 20μ thick were stained by Nissl's method and examined under the microscope. An example of selective destruction is shown in Fig. 1.

EXPERIMENTAL RESULTS

After injury to NRM or NRD incorporation of labeled precursor into proteins was discovered in the motor area of the neocortex, hippocampal area CA_3, and the head of the caudate nucleus (Fig. 2, Table 1). The most significant disturbances after destruction of NRM were found in the motor area of the neocortex and hippocampal area CA_3. In both these structures the inten-

TABLE 1. Incorporation of Leucine-^{3}H into Rat Brain Proteins after Destruction of Medial and Dorsal Raphe Nuclei (M ± m)

Protein fraction	Motor area of neocortex			Hippocampal area CA_3			Head of caudate nucleus		
	control	destruction of NRM	destruction of NRD	control	destruction of NRM	destruction of NRD	control	destruction of NRM	destruction of NRD
HMWP	0,098±0,029	0,134±0,028*	0,090±0,041	0,055±0,013	0,106±0,027*	0,110±0,025*	0,060±0,018	0,114±0,022*	0,116±0,047*
LMWP									
40 000±3000	0,050±0,010	0,090±0,012*	0,076±0,025*	0,058±0,028	0,076±0,020	0,069±0,014	0,063±0,028	0,099±0,013*	0,095±0,037*
20 000±3000	0,054±0,015	0,193±0,070*	0,079±0,039	0,040±0,004	0,088±0,030*	0,052±0,018	0,064±0,007	0,076±0,019	0,082±0,043

Legend. Interval assessment of regression coefficient shown. Significance of differences between values at $P < 0.05$ level indicated by asterisk.

sity of *de novo* synthesis both of HMWP, which includes the main mass of cytoplasmic cell proteins, and of LMWP, consisting chiefly of proteins with high relative anodal mobility during electrophoresis in 7% PAG, identical with mobility of acid neurospecific protein S-100, was observed. The increase in the level of the latter fraction was much greater, especially in the cortex. Injury to NRD was followed by less marked changes in protein biosynthesis in the motor area of the neocortex and the hippocampus.

The results suggest that the serotoninergic innervation projected from NRM plays a more important role in protein metabolism of the motor area of the neocortex and hippocampus. These results correlate with those of morphological studies, indicating predominant innervation of these structures from NRM [10, 11]. Meanwhile the increase in the level of synthesis of the various protein fractions in the head of the caudate nucleus was equally marked in the animals with destruction of NRD and of NRM. The increase in protein-synthesizing activity in this case was greater for HMWP and for the protein fraction with mol. wt. of 40,000 ± 3,000.

The difference between the effects of destruction of NRM and NRD on protein synthesis was thus greater in the cortex and hippocampus than in the caudate nucleus.

It can tentatively be suggested that the region of the head of the caudate nucleus receives a dense innervation from both nuclei, for destruction of each of them separately leads to equivalent changes in the level of protein synthesis in this brain structure. Evidence of the validity of this hypothesis is given by the results of morphological studies which show that, despite the specificity of connections of NRM and NRD, they both project to the corpus striatum [1]. Despite the great difference between its behavioral effects, destruction of NRD and NRM may have a similar effect on catecholamine metabolism in the corpus striatum [7, 12].

The cause of the changes in the level of protein biosynthesis observed after injury to the raphe nuclei may perhaps be a significant fall in the serotonin level in the brain structures studied [13, 15]. We know from the literature that serotonin blocks synthesis both of total cytoplasmic proteins [3] and of proteins of brain synaptosomes [4, 14]. It must also be pointed out that after destruction of the raphe nuclei, on account of removal of tonic inhibitory influences from the serotoninergic system, catecholamine metabolism is intensified in the above-mentioned structures [12], and this disturbs the balance between the regulatory influences of the serotoninergic and noradrenergic systems. One result of this disturbed balance may be predominance of the tonic influence of the adrenergic innervation on brain structures, accompanied by activation of biogenesis of cell proteins. The validity of this hypothesis is confirmed by the results of the writers' previous investigations [5].

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