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BRIEF COMMUNICATION

Hippocampal Activation and Incorporation of Macromolecule Precursors[1]

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JORK, R., B. LÖSSNER AND H. MATTHIES. *Hippocampal activation and incorporation of macromolecule precursors.* PHARMAC. BIOCHEM. BEHAV. 9(5) 709–712, 1978.—The effect of rhythmic slow wave activity (theta rhythm) on the incorporation of ^{3}H-leucine and ^{3}H-fucose into the total proteins of different hippocampus areas was studied. The theta rhythm was elicited by electrical stimulation of medial septum nuclei. An increase in ^{3}H-leucine incorporation into the total proteins of CA 3 and CA 1 sectors of the hippocampus was observed, whereas the stimulation had no influence on precursor incorporation into the complex CA 4/area dentata. In contrast to these findings ^{3}H-fucose incorporation into hippocampal proteins was not influenced by electrical stimulation of the medial septum. These findings are discussed in comparison to the results obtained in a learning experiment, which revealed an increased incorporation of both leucine and focuse into hippocampal proteins.

NUMEROUS studies using various methods have recently established the essential role of the hippocampus in learning and memory processes [4, 9, 22, 23]. Biochemical and microautoradiographic studies indicate training-related changes in the incorporation of precursors into RNA [11,18], proteins [10, 15, 16] and glycoproteins [14] of the rat hippocampus occur during the acquisition as well as at different times during the consolidation of a shock-motivated brightness discrimination. However, these observed alterations might be due to increased neuronal activity during the learning procedure and may not reflect qualitative changes in the course of plastic reconstructions of the neuronal network in the hippocampus. For this reason, we studied the incorporation of ^{3}H-leucine and ^{3}H-fucose into proteins and glycoproteins, respectively, after an activation of the hippocampal structure by septo-hippocampal afferents. The electrical stimulation of the medial septum, from which originate monosynaptic [8,17] and cholinergic [5] fibers to hippocampal neurones, is capable of inducing rhythmic slow wave activity in the hippocampus [3,24]. The incorporation of ^{3}H-leucine and ^{3}H-fucose into the total proteins of the hippocampal sectors CA 3, CA 1 and the complex CA 4/area dentata was assayed under these conditions.

METHOD

All experiments were carried out using male Wistar rats from our own breeding stock weighing 210–240 g. One week prior to onset of experiments the animals were anaesthetized with hexobarbital-urethane (100 resp. 600 mg/kg) and a bipolar stimulation electrode was implanted (1.7 mm AP; 1.6 mm lateral; 5.8 mm deep; 18° angle to the medial plane); hippocampal recording electrode (3.5 mm AP; 3.3 mm lateral; 2.5 mm deep); intraventricular microcannula for application of radioactive precursors (0.25 mm AP; 1.5 mm lateral; 3.5 mm deep). Stimulation and recording electrodes were made from teflon-insulated steel wire. All experiments were performed on freely moving rats. Before starting the experiments in each case the individual stimulus intensity was determined which led to occurrence of a maximal rhythmic slow wave activity in the hippocampus, the necessary voltage being 1–5 V. The stimulation was performed employing rectangular pulses of 0.5 msec duration and 7 Hz frequency for 10 min. Hippocampal EEG and orienting behavior of the animals were monitored simultaneously. The sham prepared control animals were not electrically stimulated. The labeled precursors were dissolved in artificial cerebrospinal fluid and were injected intraventricularly as 100 μl L- 4,5- ^{3}H-leucine (specific activity 58 Ci/mmol; Radiochemical Centre, Amersham, Great Britain) or 100 μCi L- 1-^{3}H-fucose (specific activity 2 Ci/mmol; Radiochemical Centre, Amersham, Great Britain) at a volume of 10 μl. The incorporation time for ^{3}H-leucine and ^{3}H-fucose was 15 and 120 min, respectively. After decapitation the brains were rapidly removed and the left hippocampus dissected [12]. Using a cutting machine [21] 1 mm thick slices were prepared. Thereafter the subregions CA 3, CA 1 and the complex CA 4/area dentata were dissected out [6]. The tissue pieces of individual hippocampus sub-regions were pooled and homogenized. After precipitation the trichloracetic acid insoluble proteins were washed and dissolved in Hyaminehydroxide (New England Nuclear). The insoluble protein readioactivity as well as that

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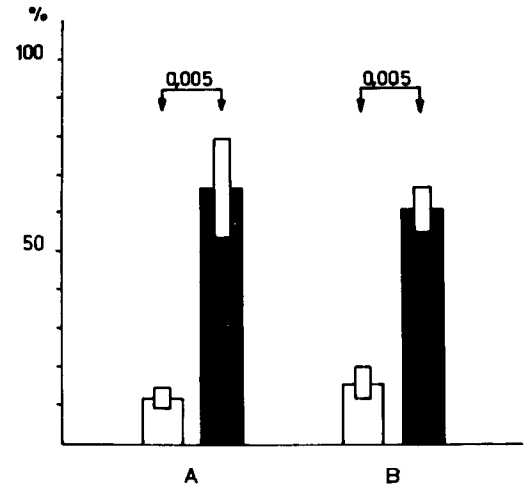

FIG. 1. Amount of theta rhythm (A) and orienting behavior (B) of stimulated animals (black columns) and controls (white columns). Using 10 min of electrical stimulation of the medial septum nuclei a slow wave rhythmical activity (theta rhythm) in the hippocampus of rats was elicited. Hippocampal EEG and behavior of the animals were monitored simultaneously. Statistical significance is based on the U-test of Mann and Whitney [20].

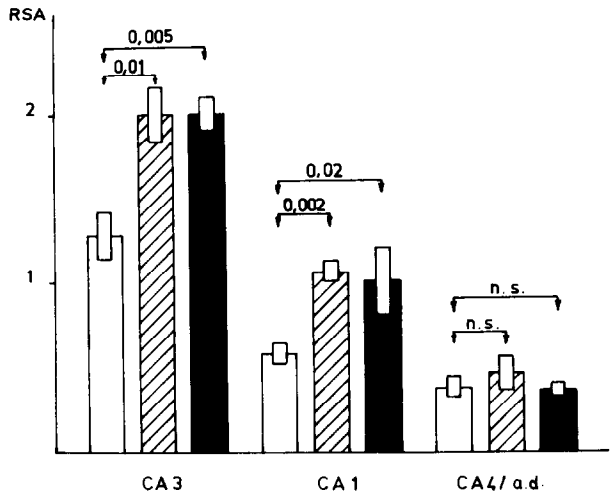

FIG. 2. Incorporation of ^{3}H-leucine expressed as relative specific activity (RSA) into total proteins of different hippocampal sectors during (shaded columns) and 45 min after (black columns) septal stimulation compared with controls (white columns). ^{3}H-leucine was intraventricularly injected as 100 μCi five min prior to and 30 min after 10 min of septal electrical stimulation. The incorporation time was 15 min. Statistical significance is based on the U-test of Mann and Whitney [20]. CA 3: CA 3 sector; CA 1: CA 1 sector; CA 4/a.d.: complex of sector CA 4+area dentate.

TABLE 1

INCORPORATION OF ^{3}H-LEUCINE INTO TOTAL PROTEINS OF DIFFERENT HIPPOCAMPAL SUB-REGIONS OF CONTROLS AND STIMULATED ANIMALS

Hippocampal sub-region		Controls			^{3}H-leucine injection 5 minutes prior to septal stimulation			^{3}H-leucine injection 30 minutes after septal stimulation		
		SAP	RFP	RSA	SAP	RFP	RSA	SAP	RFP	RSA
CA 3	$\bar{x}$	171.90	130.15	1,28	415.36	209.94	2,00	212.27	107.02	2,01
	±SEM	34.77	21.39	0,14	167.77	71.34	0,17	18.27	12.44	0,11
	N	6	6	6	5	5	5	5	5	5
CA 1	$\bar{x}$	21.73	37.42	0,57	107.72	97.40	1,06	24.51	26.62	1,00
	±SEM	4.08	5.87	0,06	29.09	21.99	0,05	3.49	4.36	0,21
	N	6	6	6	5	5	5	5	5	5
CA 4/ a.d.	$\bar{x}$	12.35	30.67	0,39	20.58	40.07	0,46	12.52	33.15	0,36
	±SEM	3.09	3.27	0,07	8.51	13.83	0,09	2.51	3.52	0,04
	N	6	6	6	5	5	5	5	5	5

$\bar{x}$ mean value
SAP specific activity of proteins (d.p.m./mg)
RFP radioactivity of free precursor
RSA relative specific activity of proteins (ratio SAP/RFP)

of the amino acids of supernatant were separated by ion exchange chromatography (DOWEX 2×8, 200–400 mesh; SERVA Entwicklungslabor Heidelberg). Fucose radioactivity for both fractions were determined in a liquid scintillation spectrometer. The obtained values of radioactivity (d.p.m.) were corrected by the protein content of tissue homogenate. The incorporation rate was expressed as relative specific activity (RSA), i.e., the ratio of specific activity of proteins to the radioactivity of free, non-incorporated precursor.

RESULTS

The incorporation of labeled leucine into total proteins of hippocampal sub-regions was measured during as well as after electrical stimulation of the medial septum. For this reason 100 μCi ^{3}H-leucine were intraventricularly injected 5 min prior and 30 min after a 10 min stimulation period. During the electrical stimulation period of the septal area the amount of rhythmic slow wave activity in the hippocampal EEG accompanied by occurrence of orienting behavior was

TABLE 2

INCORPORATION OF ^{3}H-FUCOSE INTO TOTAL PROTEINS OF DIFFERENT HIPPOCAMPAL SUB-REGIONS OF CONTROLS AND STIMULATED ANIMALS

Hippocampal sub-region		Controls			^{3}H-fucose injection 15 minutes prior to septal stimulation		
		SAP	RFP	RSA	SAP	RFP	RSA
CA 3	$\bar{x}$	193.07	502.20	0,41	184.97	423.22	0,46
	±SEM	16.40	82.80	0,04	12.09	42.09	0,06
	N	5	5	5	5	5	5
CA 1	$\bar{x}$	104.39	313.41	0,34	103.44	239.48	0,39
	±SEM	8.23	38.81	0,02	15.42	64.05	0,03
	N	5	5	5	5	5	5
CA 4/ a.d.	$\bar{x}$	147.50	430.05	0,35	138.50	345.79	0,40
	±SEM	24.59	78.28	0,02	17.23	55.10	0,04
	N	5	5	5	5	5	5

Symbols as in Table 1

significantly increased over controls (Fig. 1). Upon completion of electrical stimulation both measures were at control level. As shown in Table 1 and Fig. 2, the ^{3}H-leucine incorporation into total proteins of hippocampal sub-regions CA 3 and CA 1 was markedly increased and no changes were observed for the complex CA 4/area dentata during as well as 45 min after electrical stimulation of medial septal area. In a similar experiment, the incorporation of ^{3}H-fucose during septo-hippocampal activation was also investigated. Fifteen min prior to 10 min of medial septal nuclei stimulation 100 μCi of ^{3}H-fucose was intraventricularly injected. An incorporation time of 120 min was allowed to ensure a sufficient labeling of glycoproteins [13]. During this pulse time an appreciable metabolization of the precursor was not expected [2,25]. As demonstrated in Table 2 and Fig. 3, the ^{3}H-fucose incorporation was not significantly altered in all hippocampal sub-regions investigated following electrical stimulation of the medial septum.

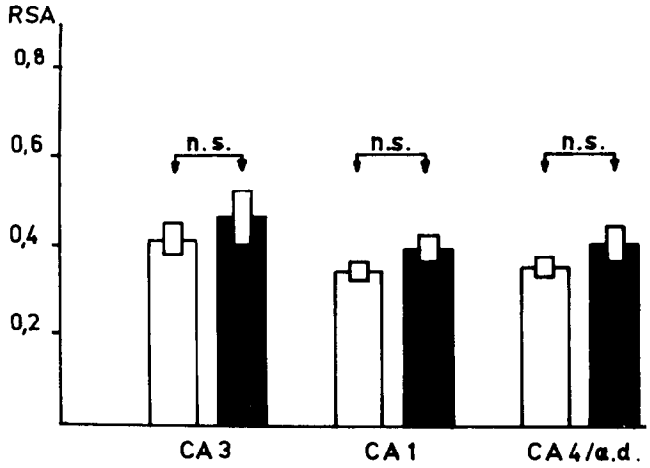

FIG. 3. Incorporation of ^{3}H-fucose into total proteins of different hippocampal sectors during electrical stimulation of medial septum nuclei of rats. ^{3}H-fucose was intraventricularly injected as 100 μCi 15 min prior to a 10-min period of the medial septal electrical stimulation which elicited theta rhythm in the hippocampus. Incorporation time was 120 min. White columns represent values of fucose incorporation obtained under control conditions and black columns represent values obtained under stimulation conditions. Symbols are as indicated in Fig. 2.

DISCUSSION

Numerous studies have provided evidence of an increased incorporation of leucine and fucose into hippocampal macromolecules during the acquisition as well as at different times during the consolidation of a brightness discrimination [10, 14, 15, 16]. The question arose as to whether these changes would be really specific to learning processes and the underlying plastic changes and not only due to an increased neuronal activity. For this purpose the incorporation of leucine and fucose was investigated under conditions of hippocampal activation by septal stimulation. During as well as after septo-hippocampal activation a considerable enhancement of leucine incorporation into total proteins of hippocampal sub-regions CA 3 and CA 1 could be observed (Table 1, Fig. 2), similar to that found during processes of acquisition and consolidation [10, 15, 16]. Contrary to the results obtained in learning experiments [14] the incorporation of fucose was not influenced by septal stimulation of the hippocampus (Table 2, Fig. 3). Thus macromolecular changes during acquisition and consolidation of a new behavior seem to differ qualitatively from those induced by simple neuronal activation. Furthermore, glycoproteins probably play a particular role in longer lasting changes of interneuronal relations, which have been assumed to occur during formation of a memory trace. If we try to characterize the functional differences between these two kinds of hippocampal manipulations, the learning procedure and the septal stimulation, the later may influence the hippocampal circuit by only one afferent system, the septo-hippocampal cholinergic link. During acquisition of a new behavior, obviously more than one afferent system would affect the hippocampus. Therefore one can speculate, that the induction of the complex macromolecular changes in the hippocampal structure by the learning procedure should be due to a spatial-temporal pattern of afferent activators that might be

mediated by different transmitter systems [7]. According to this assumption, we should expect to obtain partial components of the complex reaction during learning by the influence of different transmitter substances. Our earlier investigations seem to confirm this hypothesis [7]. The fact that leucine incorporation is altered in certain hippocampal subfields during as well as after septo-hippocampal activation might provide further evidence of their different reagibility [1,19]. These results are also indicative of a different functional significance of the different steps in the intrahippocampal circuit and will be the subject of further investigations.

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