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Effect of Serotonin on Y-Maze Retention and Hippocampal Protein Synthesis in Rats

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WETZEL, W., V. M. GETSOVA, R. JORK AND H. MATTHIES. *Effect of serotonin on Y-maze retention and hippocampal protein synthesis in rats.* PHARMAC. BIOCHEM. BEHAV. 12(2) 319–322, 1980.—The effect of serotonin (5-HT) on consolidation of a brightness discrimination reaction was investigated in rats. Five μg 5-HT, injected intrahippocampally immediately after training, impaired retention of the brightness discrimination tested 24 hr later. In biochemical experiments, leucine incorporation into hippocampal proteins *in vivo* was 32% inhibited by 5 μg 5-HT. Leucine incorporation into proteins of hippocampal slices *in vitro* was decreased by 5×10^{-3}M 5-HT. The results seem to support Essman's assumption that inhibition of brain protein synthesis by 5-HT may be responsible for the memory impairment. But also some other possibilities for a mechanism of 5-HT amnesia must be discussed.

Serotonin Brightness discrimination Memory consolidation Hippocampus Protein synthesis

A NUMBER of studies suggest a role for brain serotonin (5-HT) in learning and memory. Related experiments have shown that behavioral effects associated with brain 5-HT changes depend on the behavioral task and on the 5-HT alteration procedure. Thus, one-way active avoidance acquisition was improved by parachlorphenylalanine [2,27], but retarded by midbrain raphe lesion [13], whereas two-way active avoidance learning was facilitated by midbrain raphe lesion [12] and impaired by parachloroamphetamine [18]. Using discrimination and maze learning procedures, no effects of parachloroamphetamine or midbrain raphe lesion on Y-maze discrimination [21], and no effects of parachlorphenylalanine on food rewarded U-maze alternation [16] were found. On the other hand, discriminative operant conditioning was accelerated by parachlorphenylalanine [17] and T-maze learning ability was decreased by 5-hydroxytryptophan [32].

Compared to the numerous findings on 5-HT and acquisition of new behavior there are only a few studies concerning the role of 5-HT in memory consolidation and to our knowledge, only passive avoidance behavior was used in such studies [3,6,7,10,20]. Recently, Kruglikov *et al.* [11] found an inverse correlation between 5-HT content in the rat hippocampus and retention of a brightness discrimination. In the present study, we investigated the effect of post-training intrahippocampal 5-HT application on retention of brightness discrimination in rats. With these experiments we continued a series of studies on the involvement of different hippocampal transmitter systems in memory consolidation [15].

The second part of our study was done to compare the behavioral results with 5-HT effects on leucine incorporation into proteins of the hippocampus *in vivo* and of hippocampal slices *in vitro*.

METHOD

Animals

Sixty-five adult male Wistar rats weighing 190–240 g were used. They were housed under standard laboratory conditions in groups of ten animals per cage (for the *in vitro* experiments) or in single cages (after implantation of chronic microcannulae), respectively, with food and water ad lib.

Behavioral Procedure

One week before the experiment, chronic microcannulae were implanted into both dorsal hippocampi according to the coordinates: AP −3.1 mm; lateral 3.1 mm; vertical 3.1 mm [24]. The learning task was a foot-shock reinforced brightness discrimination reaction using a semi-automatic Y-maze [19]. Rats were trained to avoid the dark alley of the maze. Thus, the illuminated alley was the goal area, whereas runs into the dark alley (errors) were punished by 1-mA footshocks. A mean intertrial interval of 1 min was used. The training session was completed after 31 runs. Retention of the brightness discrimination was tested 24 hr after training using a relearning procedure. For evaluation of results, number of training errors and number of relearning errors were used to calculate the % savings.

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Five μg 5-HT creatinine sulfate dissolved in 1 μl artificial cerebrospinal fluid (ACF) was injected intrahippocampally immediately after the training session. Control animals received 1 μl ACF per hippocampus.

Biochemistry

For the *in vivo* studies, chronic microcannulae were implanted in the same way as for the behavioral experiments. Five minutes after intrahippocampal application of 5 μg 5-HT creatinine sulfate, 30 μCi L-4,5-^{3}H-leucine (specific activity 58 Ci/mmol; Radiochemical Centre Amersham, UK) was injected into both hippocampi at a volume of 1 μl. Controls received 1 μl ACF per hippocampus. Fifteen minutes after application of the radioactive precursor the animals were killed.

After homogenization of the dorsal hippocampus, the total proteins were precipitated using ice-cold trichloracetic acid. The precipitate was washed, delipitated, and dissolved in Hyamine hydroxide. The amino acids of supernatant were separated by ion exchange chromatography (DOWEX 2×8; 200–400 mesh; SERVA, Heidelberg). The radioactivity of both precipitate and amino acids of supernatant was determined in a liquid scintillation spectrometer and d.p.m. values were corrected by protein content of homogenate. The incorporation rate was expressed as relative specific activity (RSA), i.e., the ratio of specific activity of proteins to radioactivity of "free", non-incorporated precursor.

In vitro studies were carried out using hippocampal slices of 0.5 mm thickness prepared by cutting a hippocampus perpendicularly to the longitudinal axis [28]. After preincubating the slices 15 min in 0.8 ml of a medium containing 134 mM NaCl, 16 mM $NaHCO_3$, 0.75 mM $CaCl_2$, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$, and 10 mM glucose, pH 7.35, 5-HT creatinine sulfate at different concentrations was added to the medium at a volume of 100 μl. The incubation medium of control slices was refilled to a volume of 0.9 ml. 10 min later, 3 μCi DL-1-^{14}C-leucine (specific activity 24 mCi/mmol; Amersham, UK) at a volume of 100 μl were added to the incubation media; the precursor concentration of the medium was 10^{-3}M [4]. Incorporation time was 60 min. The slices were aerated with carbogen (95% O_2 and 5% CO_2). For calculation of the incorporation rate IR (nmoles/mg protein/hour) the specific activity of the precipitated and washed (6% trichloracetic acid; water; ethanol-ether, 1:1 v/v) proteins (d.p.m./mg protein) was corrected by the amount of radioactive precursor of incubation medium using the following equation:

$$IR = \frac{\text{d.p.m.} \times \text{mg protein}^{-1} \times \text{h}^{-1}}{\text{d.p.m.} \times \text{nmoles precursor}^{-1}\,\text{(medium)}}$$

This calculation is based on the consideration that within a few minutes the specific activity (d.p.m./nmoles precursor) of the intracellular compartment will be identical with that of the medium [4].

Statistical Analysis

The Mann-Whitney U test (for behavioral and *in vivo* biochemical results) and the Wilcoxon matched pairs signed rank test (for *in vitro* biochemical results) were used for statistical evaluations.

RESULTS

Brightness Discrimination

The effect of post-training intrahippocampal injection of 5 μg 5-HT creatinine sulfate per hippocampus on retention of brightness discrimination is shown in Table 1. Number of training errors was the same for control group and 5-HT group. However, 5-HT treated animals exhibited more relearning errors than controls, resulting in significantly fewer % savings.

TABLE 1

EFFECT OF POST-TRAINING INTRAHIPPOCAMPAL INJECTION OF 5 MICROGRAM 5-HT CREATININE SULFATE PER HIPPOCAMPUS ON RETENTION OF BRIGHTNESS DISCRIMINATION

Group	n	Training errors	Relearning errors	% savings
Control	9	10.5 (0.7)	5.2 (0.6)	51.2 (2.9)
5 μg 5-HT creatinine sulfate	11	10.6 (0.7)	7.3 (0.9)	32.3* (5.7)

Mean values; SEM in parentheses.
*$p<0.05$.

TABLE 2

EFFECT OF INTRAHIPPOCAMPAL INJECTION OF 5 MICROGRAM 5-HT CREATININE SULFATE ON INCORPORATION OF ^{3}H-LEUCINE INTO PROTEINS OF DORSAL HIPPOCAMPUS IN VIVO

Group	n	Left hippocampus	Right hippocampus
Control	4	0.80 (0.13)	0.69 (0.23)
5 μg 5-HT creatinine sulfate	4	0.54* (0.04)	0.47 (0.02)

Mean values of relative specific activity of proteins (RSA); SEM in parentheses.
*$p<0.05$.

Leucine Incorporation

The *in vivo* incorporation of ^{3}H-leucine into proteins of the dorsal hippocampus is shown in Table 2. Intrahippocampal application of 5 μg 5-HT creatinine sulfate resulted in a decreased leucine incorporation.

To study the influence of 5-HT on leucine incorporation *in vitro*, hippocampal slices were incubated in the presence of different concentrations of 5-HT creatinine sulfate. As shown in Table 3, 5-HT at concentrations of 5×10^{-3}M and 7.5×10^{-3}M led to a decreased incorporation of ^{14}C-leucine into proteins of hippocampal slices.

DISCUSSION

The involvement of 5-HT in memory consolidation was shown by several authors using passive avoidance experiments [3, 6, 7, 10, 20]. The present results show that the consolidation of a more complex learning task, a bright-

TABLE 3

INFLUENCE OF DIFFERENT CONCENTRATIONS OF 5-HT CREATININE SULFATE ON INCORPORATION OF ^{14}C-LEUCINE INTO PROTEINS OF HIPPOCAMPAL SLICES IN VITRO

5-HT Conc. (M)	n	% IR†	
10^{-5}	7	6.35	(18.36)
10^{-4}	6	−17.45	(7.57)
10^{-3}	11	−13.32	(10.13)
5×10^{-3}	6	−48.20*	(3.40)
7.5×10^{-3}	7	−57.01*	(4.00)

*$p<0.05$.

†Mean values, expressed as percentage difference of incorporation rate IR (nmol/mg protein/hr) between 5-HT treated slices and controls; SEM in parentheses.

ness discrimination, can be impaired by post-training intrahippocampal application of 5-HT. In our previous investigations, application of different neurotransmitter agonists (oxotremorine, apomorphine, clonidine, orciprenaline) was followed by a retention improvement [15]. 5-HT was the first transmitter leading to an impairment of memory retention. Conversely, in further experiments using the same behavioral method we found an improvement of memory by mianserin, a substance with 5-HT receptor blocking properties [14, 30, 31]. In these experiments, the mean number of relearning errors for the mianserin treated group (5 μg mianserin hydrochloride intrahippocampally immediately after training) was 3.8 and for the control group (1 μl distilled water) 6.0 ($p<0.002$) resulting in 61.2% and 43.9% savings ($p<0.02$), respectively, whereas no difference was in the number of training errors (10.2 and 10.9, respectively).

According to Essman [6], an inhibition of brain protein synthesis by 5-HT (see also [9]) may be responsible for the memory impairment following 5-HT application. Our biochemical results seem to support such a suggestion. The question arose as to whether the changes in incorporation would be really due to a decreased protein synthesis. For this purpose, the incorporation of leucine under the influence of 5-HT was studied *in vitro* using hippocampal slices. On the basis of a precursor concentration of 10^{-3}M an identical specific activity of precursor of the intracellular compartment and of the medium is assumed [4]. Thus, a decreased incorporation of leucine due to a decreased accumulation of the precursor in the cell can be ruled out. The decrease in leucine incorporation into proteins of hippocampal slices *in vitro* could indicate a decreased protein synthesis in the hippocampus also *in vivo*, caused by the injection of 5 μg 5-HT.

Comparing the amount of protein synthesis inhibition by 5-HT with the effects of amnesic protein synthesis inhibitors [1,8], the question arises: Why is such a relatively low amount of protein synthesis inhibition by 5-HT (32% in our experiments) followed by an impairment of memory? And on the other hand, why can rat brain protein synthesis be inhibited to some degree by d-amphetamine [22] or strychnine (unpublished results), both memory-improving stimulants? One may speculate that some special proteins, possibly important for memory consolidation, might be inhibited by 5-HT.

However, some other possibilities for a mechanism of 5-HT amnesia must also be discussed. Thus, an electrophysiological inhibition of hippocampal cells [23] and a decrease of hippocampal theta by 5-HT [26] or a suppression of REM sleep by 5-HT increase [5, 25, 29, 33] may be related to the amnesic effect.

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