Pharmacology Biochemistry & Behavior, Vol. 4, pp. 225–229.

Influence of Uridine-5-Monophosphate on ^{3}H-Leucine Incorporation into Hippocampal Neurons During Learning[1]

W. POHLE AND H. MATTHIES

Institute of Pharmacology and Toxicology, Medical Academy
301 Magdeburg, G.D.R.

(Received 8 July 1975)

POHLE, W. AND H. MATTHIES. *Influence of uridine-5-monophosphate on ^{3}H-leucine incorporation into hippocampal neurons during learning.* PHARMAC. BIOCHEM. BEHAV. 4(3) 225–229, 1976. – The influence of UMP (injected intraventricularly) on the incorporation of ^{3}H-leucine (injected intraperitoneally) was studied in rats using the microautoradiographic technique. Under the conditions of a brightness discrimination, the incorporation of labeled leucine into hippocampal neurons was significantly increased by UMP pretreatment, whereas under control conditions UMP caused only a tendency of increase without any statistical significance. It is suggested, that under learning conditions UMP substitution increases RNA synthesis, which would yield an enhanced protein synthesis.

UMP Protein synthesis Learning Hippocampus Neurons Microautoradiography

DURING and after acquisition of a shock-motivated brightness discrimination, we observed in histoautoradiographic studies an increased incorporation of labeled leucine into neurons of hippocampus and distinct cortical structures [22]. Biochemical investigations showed an increased incorporation of ^{14}C-leucine into the proteins of the corresponding brain regions in identical behavioral experiments [10]. Furthermore, we found an acceleration of the consolidation and a prolongation of the retention of the brightness discrimination as well as of other learned behaviour of rats after application of orotic acid, uridine monophosphate and uridine [12, 13, 15–19]. These substances are markedly incorporated into neuronal RNA during acquisition [1, 3, 21, 28]. Pyrimidine nucleotides seem to have relatively low concentrations in central neurons of adult rats thus probably playing a limiting role in particular functional conditions for RNA synthesis and the subsequent formation of proteins [6]. The application of these RNA precursors may overcome the limitated endogenous supply thus facilitating the macromolecular synthesis assumed to be of importance for the consolidation of a memory trace.

In order to verify this assumption, we investigated by histoautoradiography, 1) if the application of a pyrimidine nucleotide, UMP, may influence the incorporation of ^{3}H-leucine into neurons of the hippocampus of quiet control rats, and 2) if the application of the same nucleotide may facilitate not only the consolidation and retention of the brightness discrimination, as observed in our previous investigations, but also the incorporation of the labeled amino acid during the acquisition of this new behaviour.

METHOD

Male Wistar rats of identical breeding stock, 11–12 weeks old, with a body weight of 160–180 g were used for this investigation. Three days prior to the experimental procedure, a small piece of skin was removed to recognize the sutures for an exact localization of the site of injection. 100 μg uridine monophosphate in 20 μl artificial cerebrospinal fluid (ACSF) [14] or the same volume of ACSF were injected into the right ventricle. The coordinates of the injection were 0.25 mm caudal to the bregma and 1.6 mm lateral to the middle line. The exact depth of 3.5 mm was ensured by a special shape of a N°20 needle. The injection was given under light ether anesthesia 30 min prior to training. This procedure was proved not to influence the acquisition. The exact site of injection was checked histologically before the brain was taken for further autoradiographic investigation; animals with incorrect injections were discarded.

The animals were divided into 4 groups according to the experimental treatment: (1) 100 μg UMP intraventricularly 30 min prior to training; (2) ACSF intraventricularly 30 min prior to training; (3) 100 μg UMP intraventricularly without training; (4) ACSF intraventricularly without training.

Each experiment consisted of 1 rat of the same weight from each group, 7 experiments with correct injections of all animals were used for histoautoradiographic investigation. All experiments were performed at the same day time to exclude the influence of diurnal rhythms.

Upon completion of training, DL-^{3}H-leucine (specific activity 30 Ci/mmole, Institute of Isotopes, Budapest, Hungary) was applied intraperitoneally in a dose of 50

[1] This work was sponsored by the Ministry of Science and Technology of the G. D. R.

μCi/0.01 ml/g body weight to the trained and the corresponding untrained rats. The animals were decapitated 1 hr after injection of the labeled precursor, the brains were removed and fixed in formaldehyde within 30 sec. The 4 brains of each experiment were jointly dehydrated in ethanol and embedded in the same paraffine block to guarantee the identity of the following histoautoradiographic procedures for the slices of one experiment and to enable the comparison of the autoradiographs. Before embedding, the brains were carefully cut under visual control, so that all sections would represent comparable areas. Slices 5 μ thick were coated with ORWO K5 emulsion (VEB Filmfabrik Wolfen, G.D.R.), exposed for 6 weeks and developed during 3 min at 16°C in ORWO MH 28 diluted with water (1:4). Toluidine blue was used for counterstaining. Photographs were taken from 2 different slices of each animal showing comparable structures and free from artifacts. Identical morphological structures were photographed using a standard schedule previous by described [21,22]. The microphotographs were coded for blind evaluation. Forty cells of each type of cells under investigation (pyramidal cells from CA1, CA2, CA3 and CA4 sectors of hippocampus as well as granular cells of area dentata) showing an optimal cutting level, were selected randomly and the silver grains counted. The mean number of silver grains of 40 cells per cell type per animal was therefore taken from each of the 7 experiments for statistical evaluation. Using the Wilcoxon test [29,30], the following groups were compared: 1 vs. 2, 3 vs. 4, 1 vs. 3, 2 vs. 4, and 1 vs. 3/2 vs. 4.

The shock-motivated brightness discrimination [16] was trained in an Y chamber. The animals were allowed to adapt them to the start box for 5 min. After this time, the swinging door of the start box was opened and a foot shock was applied. The escape into the dark arm of the Y chamber, which corresponds to the innate behaviour of the rats, was punished by a 1 mA foot shock via the grid floor in order to learn to avoid the dark. After arriving at the illuminated arm (5 W lamp), the rats were allowed to stay there for 20 sec and were then removed to the start box. The interval between 2 runs lasted about 1 min. After 3 runs, the side of illumination and punishment were changed to prevent a direction training. The learning criterion was 16 correct runs without error including at least 5 correct responses immediately after direction change of illumination. This criterion was reached after 40–50 runs, so that the training session lasted 40–50 min. In previous experiments [22] no significant differences between active and passive controls occured, indicating only a slight influence of the footshock and locomotion. Therefore we used only passive controls in this investigation. Moreover the present study was undertaken mainly to examine the effect of UMP treatment on quiet animals and on rats under learning conditions.

RESULTS

The results are summarized in Fig. 1. The intraventricular application of UMP 75 min before the intraperitoneal administration of ^{3}H-leucine showed only a tendency of increasing the incorporation of labeled material into hippocampal neurons in the following 60 min. Only CA1 cells revealed a significant increase in incorporation in quiet animals.

On the contrary, in the trained animals the leucine incorporation was significantly increased into CA1, CA2 and CA3 cells as well as into the granular cells of area dentata during the first 60 min after acquisition by the pretreatment with UMP. The increase ranged between 20 and 35%.

In agreement with previous findings [22], learned untreated rats incorporated more lebeled leucine than the corresponding untreated passive controls. The difference was significant in all structures under investigation.

After pretreatment with UMP, the training-induced increase in incorporation was greatly enhanced in comparison with the pretreated passive controls, the differences being significant in all investigated types of cells from the hippocampus. The comparison of the increase of incorporation induced by learning in treated rats versus untreated animals (Fig. 1. statistical significance level E.) showed increases only in area dentata, CA4 and CA1 sectors of the hippocampus, the difference in area dentata being statistically significant ($p<0.02$).

DISCUSSION

An enhancement of incorporation of labeled amino acids during a learning experiment was observed by many authors using different tasks [2, 4, 5, 7, 9, 22, 23, 25, 26]. Some others failed to detect any significant changes in incorporation [8, 24, 27], so that this subject seems to be still controversial.

The interpretation of incorporation changes during learning is also a subject of discussion. The increase in incorporation of labeled amino acids in autoradiographic and biochemical experiments may only support the assumption of an increased protein synthesis involved in memory formation. But the results are not conclusive, if the specific activity of the intraneuronal precursor pool, was not determined. We observed, however, in electron microscopic studies a significant increase in membrane-bound ribosomes in the same type of hippocampal neurons of learned rats [30], which exhibited under identical experimental conditions a significant increase in the incorporation of labeled leucine [22]. Therefore we may be right to assume an increased protein synthesis during and immediately after acquisition of a learned behavior, which succeeds a corresponding quantitative or/and qualitative change in RNA synthesis.

In order to facilitate the RNA synthesis during learning, we treated rats with different RNA precursors prior to training and found that mainly pyrimidine nucleotides and their precursor orotic acid accelerate the consolidation and prolong the retention of a brightness discrimination, and other learned behaviour to a considerable degree [12, 13, 15, 16–19]. These effects were blocked by cycloheximide indicating their realization via protein synthesis [20]. We also observed that the enhanced incorporation of labeled guanosine into RNA of hippocampal neurons during the acquisition of a brightness discrimination in rats was further increased by pretreatment with UMP [28]. Summarizing the results of these behavioral, biochemical and autoradiographic studies, we assumed that the endogenous supply of pyrimidine nucleotides may limit the RNA synthesis, at least under particular functional demands, as it was supposed much earlier by Mandel [6], who found a very low level of pyrimidine nucleotides in the brain of adult rats. Therefore, the administration of these precursors may facilitate the RNA synthesis, induced during learning, but

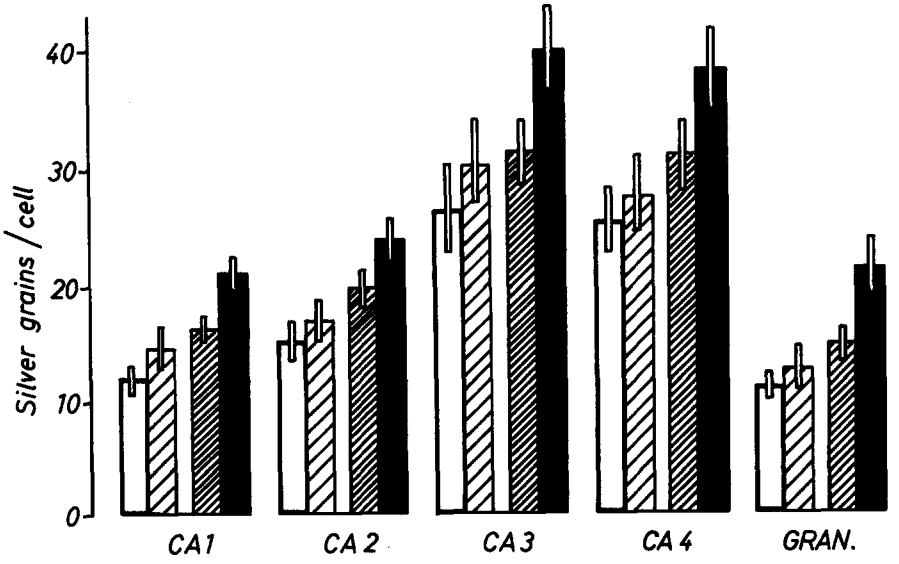

FIG. 1. Effect of UMP on ^{3}H-leucine incorporation into neurons of hippocampus formation under control conditions and after brightness discrimination.

control
training
control with UMP pretreatment
training with UMP pretreatment

CA1, CA2, CA3 and CA4 = pyramidal cells of hippocampus sectors
GRAN = granular cells of area dentata
Abscissa: Silver grains/cell ± SEM

STATISTICAL SIGNIFICANCE LEVELS (WILCOXON TEST)

		CA 1	CA 2	CA 3	CA 4	GRAN
A)	training + UMP / training	+ 36% $p<0.05$	+ 21% $p<0.02$	+ 29% $p<0.05$	+ 24% $p = 0.05$	+ 31% $p<0.05$
B)	control + UMP / control	+ 25% $p<0.05$	+ 22% n.s.	+ 28% n.s.	+ 11% n.s.	+ 15% n.s.
C)	training + UMP / control + UMP	+ 39% $p<0.02$	+ 35% $p<0.02$	+ 26% $p<0.02$	+ 39% $p<0.05$	+ 49% $p<0.02$
D)	training / control	+ 26% $p<0.02$	+ 35% $p<0.05$	+ 24% $p<0.05$	+ 26% $p<0.05$	+ 29% $p<0.05$
E)	increase in "C" / increase in "D"	$p<0.10$	n.s.	n.s.	n.s.	$p<0.02$

may also indirectly enhance the subsequent protein synthesis involved in the consolidation of a memory trace. The present results seem to support further this hypothesis. While the treatment with the pyrimidine nucleotide UMP led only to a tendency of an increase in incorporation of labeled leucine into hippocampal neurons in quiet rats, a considerable increase in leucine incorporation into hippocampal structures was observe, if the trained animals were pretreated with pyrimidine nucleotides. This incorporation was increased to a higher degree than the enhancement in

incorporation for untreated animals. These autoradiographic findings are confirmed by biochemical investigations: The increased incorporation of ^{14}C-leucine into the brain proteins of trained rats was significantly enhanced by intraventricular injection of 100 μg UMP before training [10,23]. Recently we reported that a significant increase in leucine incorporation after the acquisition of a brightness discrimination occurred not only in comparison with quiet untrained animals, but also in comparison with the active controls, which received the same number of stimuli and performed the same number of runs as the trained animals, but without learning [10,22].

It seems very unlikely that the effect of the pyrimidine nucleotides and orotic acid may be due to an influence on the permeation of labeled leucine and the resulting specific activity of the intracellular leucine pool, because this influence should be expected both in quiet and trained animals.

Some problems have to be considered with regard to the nucleotide application and the particular effect of uridine nucleotides. Our autoradiographic studies showed that, after intraventricular application of labeled UMP, a decreasing labeling of brain structures was observed with increasing distance from the ventricle. But the gradient of labeling occurred in quiet as well as in trained animals, so that corresponding brain structures could be compared. The hippocampus was also subject of our evaluation, since the most pronounced metabolic changes during and after acquisition of the brightness discrimination occurred in this structure, even after systemic application of the labeled material [10,22]; the prolongation of retention of this learned behaviour was obtained, if uridine nucleotide was only injected into the dorsal hippocampus [19].

Some years ago, Mandel and coworkers observed relatively low concentrations of pyrimidine nucleotides (especially of cytidine) in the adult rat brain and supposed their limiting roll for the RNA synthesis in the brain [6]. We observed, however, a stronger effect of UMP in comparison with CMP, where as GMP was only slightly effective; AMP showed no effect [19]. Exact data on the steps of conversion and penetration into neuronal cells of the different nucleotides are not available with us. The unexpected stronger effect of UMP may result from the possibility that, UMP may be transformed via UTP to CTP, thus feeding the pool of cytidine nucleotides, too, which are supposed to limit the RNA synthesis in neurons and therefore, according to our working hypothesis [11], to determine the velocity and degree of consolidation of a memory trace.

ACKNOWLEDGEMENT

The skillful technical assistance of Frau Uta Riechert is gratefully acknowledged.

REFERENCES

1. Adair, L. B., J. E. Wilson, J. W. Zemp and E. Glassman. Brain function and macromolecules. III. Uridine incorporation into polysomes of mouse brain during short-term training experiences. *Proc. Natn. Acad. Sci.* (U.S.A.) **61**: 606–613, 1968.
2. Beach, G., M. Emmens, D. P. Kimble and M. Lickey. Autoradiographic demonstration of biochemical changes in the limbic system during avoidance training. *Proc. Natn. Acad. Sci.* (U.S.A.) **62**: 692–696, 1969.
3. Bowman, R. E. and D. A. Strobel. Brain RNA metabolism in the rat during learning. *J. comp. physiol. Psychol.* **67**: 448–456, 1969.
4. Hydén, H. Protein metabolism in the nerve cell during growth and function. *Acta physiol. scand. 6. Suppl.* **17**: 1–137 (1943.
5. Hydén, H. and P. W. Lange. Protein synthesis in hippocampal pyramidal cells and rats during a behavioral test. *Science* **159**: 1370–1373, 1968.
6. Jacob, M. and P. Mandel. RNA and the central nervous system. In: Protides of the biological fluids, edited by H. Peeters. Amsterdam: Elsevier Publ. Comp., 1965, pp. 63–80.
7. Kerkut, G. A., G. Oliver, I. T. Rick and R. J. Walker. Biochemical changes during learning in an insect ganglion. *Nature* **227**: 722–723, 1970.
8. Kruglikov, R. I., M. M. Aleksandrovskaya, F. A. Brasovskaya, M.Ja. Maiselis and A. S. Basyan. Гистоауторадиографическое исследование включения ^{3}H-лейцина в ядра нейронов различных образований головного мозга при выработке оборонительных условных рефлексов. *Doklady A. N. S.S.S.R.* **206**: 1470–1472, 1972.
9. Levitan, I. B., G. Ramirez and W. E. Mushynski. Amino acid incorporation in the brain of rats trained to use the nonpreferred paw in retrieving food. *Brain Res.* **47**: 147–156, 1972.
10. Lössner, B. and H. Matthies. L-leucine-1-^{14}C incorporation in total proteins of different brain regions during development of a conditioning reaction. *Pharmac. Biochem. Behav.* (in press).
11. Matthies, H. The biochemical basis of leaning and memory. *Life Sci.* **15**: 2017–2031, 1974.
12. Matthies, H., Chr. Fähse and W. Lietz. Die Wirkung von RNS-Präcursoren auf die Erhaltung des Langzeitgedächtnisses. *Psychopharmacologia* **20**: 10–15, 1971.
13. Matthies, H. and M. Kirschner. Die Wirkung der Orotsäure auf den stabsprung-Test bei der Ratte. *Acta biol. med. german.* **19**: 789–790, 1967.
14. Merlis, J. K. The effect of changes in the calcium content of the cerebrospinal fluid on reflex activity in the dog. *Am. J. Physiol.* **131**: 67–72, 1940.
15. Ott, T., B. Lössner and H. Matthies. Die Wirkung von Nucleotid-Monophosphaten auf die Akquisition und Extinktion bedingter Reaktionen. *Psychopharmacologia* **23**: 261–271, 1972.
16. Ott, T. and H. Matthies. Die Wirkung der Orotsäure auf die durch elektroconvulsiven Schock ausgelöste retrograde Amnesie. *Psychopharmacologia* **20**: 16–21, 1971.
17. Ott, T. and H. Matthies. Der Einfluss von Orotsäure und Pentetrazol auf die Akquisition und Extinktion am Modell der optischen Diskriminlierung. *Acta biol. med. german.* **26**: 79–85, 1971.
18. Ott, T. and H. Matthies. Der Einfluss von 6-Azauridin auf die Begünstigung des Wiedererlernens durch Ribonucleinsäure-Präcursoren. *Psychopharmacologia* **23**: 272–278, 1972.
19. Ott, T. and H. Matthies. Some effects of RNA precursors on development and maintenance of long-term memroy: Hippocampal and cortical pre- and post-training application of RNA precursors. *Psychopharmacologia* **28**: 195–204, 1973.
20. Ott, T. and H. Matthies. Suppression of uridine monophosphate-induced improvement in long term storage by cycloheximide. *Psychopharmacologia* **28**: 103–106, 1973.
21. Pohle, W. and H. Matthies. The incorporation of ^{3}H-uridine monophosphate into the rat brain during the training period. A microautoradiographic study. *Brain Res.* **29**: 123–127, 1971.
22. Pohle, W. and H. Matthies. Incorporation of ^{3}H-leucine into brain cells after learning. *Pharmac. Biochem. Behav.* **2**: 573–577, 1974.

23. Pohle, W., B. Lössner, H.-L. Rüthrich and H. Matthies. Incorporation of labelled precursors into brain RNA and proteins during acquisition and consolidation of a brightness discrimination. *Erg. exper. Med.* **17:** 612–625, 1974.
24. Rees, H. D., L. L. Brogan, D. J. Entingh, A.-J. Dunn, P. G. Shinkman, T. Damstra-Entingh, J. E. Wilson and E. Glassman. Effect of sensory stimulation on the uptake and incorporation of radioactive lysine into protein of mouse brain and liver. *Brain Res.* **68:** 143–156, 1974.
25. Richardson, G. and S. P. R. Rose. Changes in ^{3}H-lysine incorporation following first exposure to light. *Brain Res.* **44:** 229–303, 1972.
27. Routtenberg, A. and W. Bondareff. Protein synthesis and memory consolidation: Radioautographic study of intrahippocampal microinjections of ^{3}H-leucine in awake freely moving animals. *Fedner. Proc. Am. Soc. exp. Biol.* **30:** 215, 1971.
28. Rüthrich, H. L., W. Pohle and H. Matthies. Increase of guanosine incorporation into RAN of hippocampal neurons by application of uridine monophosphate during a learning experiment. *Brain Res.* **69:** 49–55, 1974.
29. Siegel, S. Nonparametric statistics. London: Mc. Graw Hill Book Comp. 1956, pp. 254.
30. Wenzel, J., H. David, W. Pohle, I. Marx and H. Matthies. Free and membrane-bound ribosomes and polysomes in hippocampal neurons during a learning experiment. *Brain Res.* **84:** 99–109, 1975.
31. Wilcoxon, S. and R. A. Wilcox. Some rapid approximate statistical procedures. New York: Pearl River Lederle Lab., 1964.