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Apomorphine and Glycoprotein Synthesis During Consolidation

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JORK, R., GISELA GRECKSCH AND H. MATTHIES. *Apomorphine and glycoprotein synthesis during consolidation.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 11–13, 1982.—The dopamine agonist, apomorphine, was injected intrahippocampally immediately after acquisition of a brightness discrimination task, which was motivated by footshock in rats. This led to an increase in the incorporation of L-fucose into total proteins which were measured in the hippocampus 7–9 hours later. In behavioral experiments, the same application improved the retention of a learned task. A possible linkage between increased glycoprotein synthesis and improvement of the retention of a new learned behavior due to the action of apomorphine is discussed.

Rat Hippocampus Apomorphine Glycoprotein synthesis Consolidation

THE specific role of an increased synthesis of macromolecules, in changes of the synaptic efficiency underlying memory processes has been well established [5, 8, 10, 16, 17, 20, 21, 25, 28, 29, 33]. Glycoproteins are thought to be an important constituent of synaptic membranes in that the efficiency of interneuronal connections is determined to a high degree by the presence of this macromolecules [1, 3, 5, 11, 12, 20, 21, 26]. In experiments where alterations in the neuronal connectivity can be assumed there is evidence for an increase in the incorporation of L-(1-^{3}H)fucose [2, 3, 4, 13, 20, 21, 22, 26], a relative specific precursor of glycoproteins [6, 7, 33]. Thus, an elevation in glycoprotein synthesis as a consequence of a training experience has been found in the hippocampus [20,26], a structure which has been repeatedly shown to be of importance in learning and memory formation [9, 10, 18, 20, 23, 26, 31, 32]. Changes in the hippocampal glycoprotein synthesis as observed in learning experiments are similar to those elicited by the action of the dopaminergic agonist apomorphine in vivo [15] and in vitro [14]. Furthermore, in rats an intrahippocampal application of apomorphine improved the retention of a brightness discrimination task significantly [20]. Therefore a linkage between an increased glycorportein synthesis and the consolidation of a long-term memory trace within the rat hippocampus might be assumed. To prove this assumption we examined whether or not the apomorphine induced improvement of consolidation processes is accompanied by changes in the glycoprotein synthesis of rat hippocampus. Thus, in rats treated with apomorphine immediately after a brightness discrimination task the incorporation of ^{3}H-fucose into total proteins of the hippocampal structure was determined. Values of fucose incorporation were compared to those of such animals which received only an intrahippocampal control injection of the solvent after the training experiment.

METHOD

Animals

Forty-five adult male Wistar rats weighing 210–240 g were used. They were housed under standard laboratory conditions in groups of ten animals per cage and in single cages (after implantation of chronic microcannulae), with food and water ad lib. One week prior to onset of experiments the animals were anaesthetized with hexobarbital-urethane (100 resp. 600 mg/kg) and a microcannula for application of apomorphine and the radioactive labeled glycoprotein precursor was implanted into the left dorsal hippocampus (AP −3.1 mm, lateral 3.1 mm, vertical 3.1 mm) [30].

Training Procedure

The training procedure was carried out using the training model of a foot-shock motivated brightness discrimination as described earlier [24]. Initially, the animals were allowed to stay for 10 minutes in the semiautomatic Y-chamber for habituation. Thereafter by application of 1 mA current to the grid floor the animals escaped from the starting compartment and had to run in the illuminated alley of the chamber, since entering the non-illuminated alley was punished by an electric foot-shock. A run was evaluated as correct when the animals ran directly into the illuminated alley of the chamber. After every three runs the direction of the illuminated alley was changed so as to avoid position training. The

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TABLE 1

INFLUENCE OF APOMORPHINE ON FUCOSE INCORPORATION IN THE HIPPOCAMPUS OF TRAINED RATS

Treatment of trained rats		0–2 hr			2–4 hr			7–9 hr		
		SAS	SAP	RSA	SAS	SAP	RSA	SAS	SAP	RSA
Ascorbic acid	Mean	283.33	259.09	0.98	228.40	246.90	1.02	443.71	390.60	0.95
	SEM	65.11	49.35	0.06	81.11	90.90	0.10	75.91	40.72	0.06
	n	7	7	7	6	6	6	9	9	9
Apomorphine (40 μg)	Mean	202.45	198.14	1.04	229.07	226.29	0.95	298.20	338.55	1.17*
	SEM	37.71	24.25	0.08	52.07	52.97	0.08	46.28	48.29	0.06
	n	7	7	7	8	8	8	8	8	8
Percent deviation				+6.12			−6.86			+23.16*

SAP=Specific activity of total proteins (d.p.m./mg protein).
SAS=Specific activity of the supernatant (d.p.m./mg protein).
RSA=Relative specific activity of proteins (ratio SAP/SAS).
*$p<0.05$ according to the U-test of Mann and Whitney.

mean time between two runs was 60 seconds. The training sessions involved 31 runs.

Biochemical Procedure

Immediately after the training session 40 μg apomorphine dissolved in 0.1% ascorbic acid were injected in a volume of 2 μl into the left dorsal hippocampus. The control animals received an identical volume of 0.1% ascorbic acid after the training session. Also injected into the hippocampal structure of all animals were 10 μCi L-1-(^{3}H)fucose (specific activity 2 Ci/mmole: Radiochemical Centre Amersham, Great Britain) dissolved in artificial cerebrospinal fluid, at a volume of 1 μl 10 minutes, 2 hours and 7 hours, after the apomorphine or ascorbic acid injection. In each case an incorporation time of 2 hours was used. The animals were then killed and the hippocampus was dissected out [27]. Due to the position of the implanted microcannulae above the dorsal hippocampus this part of the hippocampal structure revealed the highest incorporation. Therefore, only the dorsal hippocampus was homogenized in 0.3 ml 0.1 N NaOH. From 50 μl of the homogenate the total proteins were precipitated using 0.5 ml of ice-cold 12% trichloracetic acid. After centrifugation the resulting protein pellet was washed twice with 6% trichloracetic acid, water and ethanol-ether (1:1, v:v). The proteins were dissolved in 0.5 ml Hyamine hydroxide (New England Nuclear) and their radioactivity as well as that of an aliquot of the trichloracetic acid soluble fraction was determined in a liquid scintillation spectrometer (Intertechnique, Plaisir, France) using a dioxane containing scintillator. The obtained values of radioactivity (d.p.m.) were corrected by the protein content of the tissue [19]. Since fucose was found to be incorporated in glycoproteins exclusively and not to be converted into other monosaccharides the incorporation rate was expressed as the relative specific activity of proteins to the radioactivity of the free, non-incorporated precursor.

RESULTS

Comparing the incorporation of fucose into the total proteins of the hippocampal structure of rats 0–2 or 2–4 hours after a learning task no differences were found between animals which received an injection of apomorphine and those ones which were treated with ascorbic acid immediately after the training session (Table 1). In contrast, the incorporation of the sugar was significantly increased in apomorphine treated animals when measured 7–9 hours after the training experiment (Table 1).

DISCUSSION

In learning experiments an increased incorporation of L-fucose into the glycoproteins of rat hippocampus was measured [20, 21, 22] similar to that observed under the influence of the dopamine agonist, apomorphine, in vivo [15] and in vitro [14]. Moreover, in behavioral experiments an intrahippocampal application of 40 μg apomorphine immediately after the acquisition of a brightness discrimination influenced positively the consolidation of the long-term memory trace [20]. In these experiments the same dose led to an elevation in the incorporation of fucose 7–9 hours after the learning task. Therefore, a link between an increased glycoprotein synthesis after treatment with apomorphine and an improvement of the consolidation of a new learned behavior due to the action of the drug can be assumed, supporting the involvement of glycoproteins in processes of long-term storage [2, 4, 5, 12, 13, 20, 21, 22, 26]. On the other hand in earlier studies [15] 40 μg apomorphine injected intrahippocampally to naive rats caused a decrease in the incorporation of fucose measured within 2 hours after the application of the drug whereas 5 μg had a positive effect. But both the time periods in which the incorporation of fucose was measured and the functional state of the hippocampal neurones in naive and trained animals are different. This could be an explanation for the discrepance of such doses of apomorphine leading to an increased incorporation of fucose under different experimental conditions. Therefore, in further studies the influence of diverse doses of apomorphine injected at several times after the training session on the retention of a brightness discrimination reaction will be investigated.

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REFERENCES

1. Barondes, S. H. Brain glycomacromolecules and interneuronal recognition. In: *The Neurosciences: Second Study Program,* edited by F. O. Schmitt. New York: The Rockefeller University Press, 1970, pp. 747–760.
2. Burgoyne, R. D. and S. P. R. Rose. Subcellular localization of increased incorporation of (^{3}H)fucose following passive avoidance learning in the chick. *Neurosci. Lett.* **19:** 343–348, 1980.
3. Burgoyne, R. D. and S. P. R. Rose. Changes in glycoprotein metabolism in the cerebral cortex following first exposure of dark reared rats to light. *J. Neurochem.* **34:** 510–517, 1980.
4. Damstra, T., D. Entingh, J. E. Wilson and E. Glassman. Incorporation of ^{3}H-L-fucose into brain glycoproteins during environmental stimulation following intracranial, intravenous or subcutaneous injections. *Behav. Biol.* **13:** 121–126, 1975.
5. Dunn, A. J. Neurochemistry of learning and memory: an evaluation of recent data. *A. Rev. Psychol.* **31:** 343–390, 1980.
6. Glasgow, M. S., R. H. Quarles and S. Grollman. Metabolism of fucoglycoproteins in the developing rat brain. *Brain Res.* **42:** 129–137, 1972.
7. Gombos, G., J-P. Zanetta, G. Vincendon and A. Federico. Turnover of fucosyl glycoproteins in rat brain. Abstracts of the first meeting of the European Society of Neurochemistry, Bath, England, 1976.
8. Hambley, J. W., J. Haywood, S. P. R. Rose and P. P. G. Bateson. Effects of imprinting on lysine uptake and incorporation into protein in chick brain. *J. Neurobiol.* **8:** 109–118, 1977.
9. Hyden, H. and P. W. Lange. Protein changes in nerve cells related to learning and conditioning. In: *The Neurosciences: Second Study Program,* edited by F. O. Schmitt. New York: The Rockefeller University Press, 1970, pp. 278–289.
10. Hyden, H., P. W. Lange and C. L. Perrin. Protein pattern alterations in hippocampal and cortical cells as a function of training in rats. *Brain Res.* **119:** 427–437, 1977.
11. Irwin, L. N. Glycolipids and glycoproteins in brain function. In: *Review of Neurosciences,* edited by S. Ehrenpreis and I. J. Kopin. New York: Raven Press, 1974, pp. 137–179.
12. Irwin, L. N., R. A. Baraaco and D. M. Terrian. Protein and glycoprotein metabolism in brains of operantly conditioned pigeons. *Neuroscience* **3:** 457–463, 1978.
13. Jork, R., B. Lössner and H. Matthies. Hippocampal activation and incorporation of macromolecule precursors. *Pharmac. Biochem. Behav.* **9:** 709–712, 1978.
14. Jork, R., B. Lössner and H. Matthies. Dopamine and macromolecule synthesis in rat hippocampus. *Pharmac. Biochem. Behav.* **11:** 247–249, 1979.
15. Jork, R., G. Grecksch, M. Jirka, B. Lössner and H. Matthies. Apomorphine and glycoprotein synthesis in rat hippocampus. *Pharmac. Biochem. Behav.* **12:** 317–318, 1980.
16. Krivanek, J. Labeling of TCA-soluble fraction and protein of the rat visual cortex after learning a pattern discrimination. *Experientia* **31:** 1042–1043, 1975.
17. Levitan, I. B., G. Ramirez and W. W. Mushyski. Amino acid incorporation in brain of rats trained to use the non-preferred paw in retrieving food. *Brain Res.* **47:** 147–156, 1972.
18. Lössner, B., R. Jork, M. Krug and H. Matthies. Protein synthesis in rat hippocampus during learning and stimulation experiments. In: *Proceedings of the 6th International Neurobiological Symposium on Learning and Memory. Magdegurg, 1980.* IBRO Monograph Series, edited by C. Ajmone-Marsan. New York: Raven Press, in press.
19. Lowry, O., N. J. Rosenbrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
20. Matthies, H. Learning and Memory. In: *Advances in Phamacology and Therapeutics. vol. 5. Neuropharmacology,* edited by C. Dumont. Oxford: Pergamon Press, 1978, pp. 117–135.
21. Matthies, H. Pharmacology of learning and memory. *Trends Pharmac. Sci.* **1:** 333–336, 1980.
22. Morgan, D. G. Incorporation of intrastriatally injected (^{3}H)fucose into electrophoretically separated synaptosomal glycoproteins. II. The influence of passive avoidance training. *Brain Res.* **179:** 343–354, 1979.
23. Olds, J., J. F. Diesterhoft, M. Segal, C. L. Kornblith and R. Hirsch. Learning centres of rat brain mapped by measuring latencies of conditioned responses. *J. Neurophysiol.* **35:** 202–219, 1972.
24. Ott, T., A. Doske, W. Thiemann und H. Matthies, Eine teilauthmatische Lernanlage für optische Diskriminierungsreaktionen mit Ratten. *Acta biol. med. germ.* **29:** 103–108, 1972.
25. Peters, R. I. Time-dependent results of amino acid uptake studies of learning in frogs. *Pharmac. Biochem. Behav.* **4:** 7–11, 1976.
26. Popov, N., S. Schulzeck, W. Pohle and H. Matthies. Changes in the incorporation of (^{3}H)fucose into rat hippocampus after acquisition of a brightness discrimination reaction. An electrophoretic study. *Neuroscience* **5:** 161–167, 1980.
27. Popov, N., W. Pohle, B. Lössner, S. Schulzeck, S. Schmidt, T. Ott and H. Matthies. Regional distribution of RNA and protein radioactivity in the rat brain after intraventricular application of labeled precursors. *Acta biol. med. germ.* **31:** 51–62, 1973.
28. Shashoua, V. E. and M. E. Moore. Effects of antisera to β and γ goldfish brain proteins on the retention of a newly acquired behavior. *Brain Res.* **148:** 441–449, 1978.
29. Shashoua, V. E. Brain metabolism and the acquisition of new behaviors. I. Evidence for specific changes in the pattern of protein synthesis. *Brain Res.* **111:** 347–364, 1970.
30. Skinner, J. E. *Neuroscience: A Laboratory Manual.* London: W. B. Saunders, 1971.
31. Vinogradova, O. S. Registration of information and the limbic system. In: *Short-Term Changes in Neural Activity and Behavior,* edited by G. Horn and R. A. Hinde Cambridge: University Press, 1970, pp. 95–140.
32. Yanigihara, T. and H. Hyden. Protein synthesis in various regions of rat hippocampus during learning. *Expl Neurol.* **31:** 151–164, 1971.
33. Zatz, M. and S. H. Barondes. Fucose incorporation into glycoproteins of mouse brain. *J. Neurochem.* **17:** 157–163, 1970.