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Apomorphine and Glycoprotein Synthesis in Rat Hippocampus[1]

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JORK, R., G. GRECKSCH, M. JIRKA, B. LÖSSNER AND H. MATTHIES. *Apomorphine and glycoprotein synthesis in rat hippocampus*. PHARMAC. BIOCHEM. BEHAV. 12(2) 317–318, 1980.—Apomorphine intrahippocampally injected at a dose of 5 μg led to a significant increase in incorporation of (^{3}H)-fucose into total proteins of this brain area, whereas 40 μg led to a significant decrease in comparison to controls, providing further evidence for the existence of dopaminoceptive structures in the hippocampus of rats and their significance for glycoprotein metabolism.

Rat Hippocampus Apomorphine Fucose Glycoprotein synthesis

RECENT studies provided evidence for an action of apomorphine in the hippocampus of rats [7] supporting the transmitter role of dopamine in this brain area shown by biochemical studies [1]. In our last investigations an increased incorporation of (^{3}H)-fucose into glycoproteins of hippocampal slices under the influence of dopamine and apomorphine was observed [4]. Taking into consideration the limitation of the *in vitro* system of brain slices in reflection of physiological processes [2, 5, 8], the question arose as to whether an increased incorporation of (^{3}H)-fucose into hippocampal glycoproteins can also be induced by apomorphine *in vivo*. Thus, the influence of different doses of apomorphine on incorporation of (^{3}H)-fucose into total proteins of the dorsal hippocampus of rats was investigated.

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 microcannula for application of the drug and the radioactive precursor respectively was implanted into the left and the right dorsal hippocampus (AP–3.1 mm; lateral 3.1 mm; vertical 3.1 mm) [10]. Apomorphine at different doses were dissolved in 0.1% ascorbic acid and injected as 2 μl (40 μg) and 1 μl (20; 5; 1.25 μg), respectively. The control animals received an identical volume of 0.1% ascorbic acid. Ten minutes later 10 μCi L-1-(^{3}H)-fucose (specific activity 2 Ci/mmole; Radiochemical Centre, Amersham, Great Britain) dissolved in artificial cerebrospinal fluid was injected at a volume of 1 μl. After an incorporation time of 120 min the animals were killed and the dorsal hippocampus was dissected out [9]. The tissue was homogenized in 0.3 ml of 0.1 N NaOH. Fifty μl of the homogenate were treated with 12% trichloracetic acid to precipitate the total proteins. After centrifugation the 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 were 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 homogenate [6]. The incorporation rate was expressed as relative specific activity (RSA), i.e., the ratio of the specific activity of proteins to the radioactivity of the free, non-incorporated precursor.

RESULTS AND DISCUSSION

The investigation of the influence of apomorphine on fucose incorporation into hippocampal glycoproteins was undertaken to ascertain if the increased incorporation of this precursor caused by dopamine and apomorphine using hippocampal slices [4] is also detectable *in vivo*. Thus, the effect of several doses of apomorphine on fucose incorporation was investigated. As shown in Table 1 a dose of 5 μg apomorphine caused a significant increase in fucose incorporation into total proteins of the dorsal hippocampus reflecting an increase in precursor incorportion into hippocampal glycoproteins [3,11]. These alterations are detectable in the

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TABLE 1

INFLUENCE OF APOMORPHINE ON FUCOSE INCORPORATION IN VIVO AND IN VITRO

Dose (μg)	In vivo $\bar{x}$ RSA	SEM	N	Percentage difference	Concentration (M)	In vitro $\bar{x}$ IR	SEM	N	Percentage difference Mean values	Values of pairs
Control	1.18	0.07	12	—	Control	0.40	0.02	25	—	—
1.25	1.29	0.14	3	+ 9.3	5×10^{-5}	0.44	0.05	6	+ 10.0	+ 5.0
5. 0	1.46*	0.09	6	+ 23.7	7.5×10^{-5}	0.49	0.03	7	+ 22.5	+ 34.6†
20. 0	1.07	0.13	7	− 9.3	5×10^{-4}	0.39	0.03	6	− 11.4	− 4.4
40.0	0.75*	0.10	6	− 36.4	1×10^{-3}	0.33	0.03	6	− 17.5	− 23.7†

$\bar{x}$=mean value.
RSA=relative specific activity of proteins.
IR=incorporation rate (nmoles/mg protein/hour) values are taken from ref. [4].
*$p<0.02$ according to the U-test of Mann and Whitney.
†$p<0.05$ according to Wilcoxon matched pairs signed rank test.

left as well as in the right dorsal hippocampus. 1.25 and 20 μg, respectively, did not alter the incorporation of fucose, whereas 40 μg apomorphine led to a significant decrease in the incorporation of the glycoprotein precursor. Comparing the *in vivo* and *in vitro* effects of apomorphine on fucose incorporation into hippocampal proteins a similar alteration of precursor incorporation in dependence to the concentration of the drug is detectable (Table 1). Thus the alteration in fucose incorporation under the influence of apomorphine *in vivo* as well as the induction of theta rhythm in the hippocampal EEG [7], the improvement of the retention of a foot shock motivated brightness discrimination [7] and the increase in the incorporation rate of fucose into glycoproteins of hippocampal slices [4] caused by this drug provide further evidence for the existence of dopaminoceptive structures in the rat hippocampus, their significance for glycoprotein metabolism and their probable biological relevance in adaptive processes. However, it should be mentioned that the dose of 40 μg apomorphine improving the retention of a learned brightness discrimination after intrahippocampal application [7] did not induce those alterations in fucose incorporation seen in learning experiments [7]. But this could be due to the different functional state of the hippocampal structure in the learning experiment and in this biochemical investigation, respectively, so further experiments are necessary to elucidate the different effect of several doses of apomorphine on behavioral, electrophysiological and biochemical phenomena.

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REFERENCES

1. Bischoff, B., B. Scatton and J. Korf. Biochemical evidence for a transmitter role of dopamine in the rat hippocampus. *Brain Res.* **165:** 161–165, 1979.
2. Dunlop, D. S., W. van Elden and A. Lajtha. Measurement of rates of protein synthesis in rat brain slices. *J. Neurochem.* **22:** 812–830, 1974.
3. Gombos, G., J.-P. Zanetta, G. Vincendon and A. Federico. Turnover of fucosyl glycoproteins in rat brain. *Abstr. First meet. Eur. Soc. Neurochem.*, Bath, England, 1976.
4. Jork, R., B. Lössner and H. Matthies. Dopamine and macromolecule synthesis in rat hippocampus. *Pharmac. Biochem. Behav.* **11:** 247–249, 1979.
5. Lajtha, A. and D. Dunlop. In: *Drugs and the Developing Brain*, edited by A. Vernadakis and N. Weiner. New York: Plenum Press, 1973, pp. 215–229.
6. 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.
7. Matthies, H. In: *Brain Mechanisms in Memory and Learning*, edited by M. A. B. Brazier. International Brain Research Organization Monograph Series. New York: Reven Press, 1979, Vol. 4, p. 197.
8. Pavlik, A. and B. Jakoubek. Distribution of protein-bound radioactivity in brain slices of the adult rat incubated with labelled leucine. *Brain Res.* **101:** 113–128, 1976.
9. 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 labelled precursors. *Acta. Biol. med. germ.* **31:** 51–62, 1973.
10. Skinner, J. E. *Neuroscience: A Laboratory Manual London:* W. B. Saunders, 1971.
11. Zatz, M. and S. H. Barondes. Fucose incorporation into glycoproteins of mouse brain. *J. Neurochem.* **17:** 157–163, 1970.