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The Influence of Dopamine on the Incorporation of Different Sugars into Total Proteins of Hippocampal Slices[1]

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JORK, R., B. LÖSSNER AND H. MATTHIES. *The influence of dopamine on the incorporation of different sugars into total proteins of hippocampal slices*. PHARMAC. BIOCHEM. BEHAV. 13(2) 303–304, 1980.—Dopamine increases the incorporation of L-fucose and of D-mannose to a similar significant degree, whereas the incorporation of D-galactose as well as of N-acetyl-D-glucosamin into the total proteins of hippocampal slices was only slightly enhanced. The incorporation of N-acetyl-neuraminic acid was not influenced by dopamine. The results suggest that the effect of dopamine on glycoprotein formation seems mainly to depend on the kind of nucleotides necessary for activation of sugars and not on the sugar's final position in the glycan chain.

L-fucose D-Mannose Dopamine Proteins Hippocampal slices

DURING consolidation of a memory trace in a brightness discrimination on rats, a considerable increase of incorporation of labelled L-fucose into the total proteins of the hippocampal structures can be observed [6,8], indicating an enhanced glycoprotein synthesis after acquisition of a new behavior. This enhanced glycoprotein formation has been shown to occur at two different times, immediately as well as six to nine hours after the learning procedure [6,8]. An increased incorporation of L-fucose into hippocampal proteins can be induced in vivo after intrahippocampal injection as well as in vitro on hippocampal slices by dopamine and dopaminergic agonists [3,4], substances, which do improve the retention of the learned behavior after posttrial application [6]. These results suggested a regulatory influence of dopaminergic inputs to hippocampal neurons and their glycoprotein metabolism in the course of the formation of a memory trace. In order to investigate the specifity and site of action of this influence, we compared the effect of dopamine on the incorporation of other sugars involved in the formation of glycan chains of glycoproteins, which differ by the nucleotides necessary for their activation before the glycosylation or by their final position in the glycan residue.

METHOD

For the experiments, eight-week-old male Wistar rats from our own breeding stock were used. From the removed hippocampus [7] 0.5 mm thick slices were prepared by cutting the structure perpendicularly to its longitudinal axis [9]. In each experiment 12 slices from one hippocampus were used and the incorporation of fucose and of another sugar into total proteins of three slices was determined parallely under control conditions and in the presence of 5×10^{-4} M dopamine in the incubation medium (134 mM NaCl; 5 mM KCl; 1.24 mM KH_2PO_4; 1.3 mM $MgSO_4$; 0.75 mM $CaCl_2$; 16 mM $NaHCO_3$; 10 mM glucose; pH 7.35), respectively. (For details of incubation see ref. [4]). D-(1-^{3}H)-mannose (25 μCi per incubation vial, specific activity 5 Ci/mmole), L-(1-^{3}H)-fucose (25 μCi per incubation vial, specific activity 4.6 Ci/mmole), N-(^{3}H)-acetyl-D-glucosamin (25 μCi per incubation vial, specific activity 500 mCi/mmole, D-(1-^{3}H)-galactose (25 μCi per incubation vial, specific activity 3 Ci/mmole) and N-acetyl-(4, 5, 6, 7, 8, 9-^{14}C)-neuraminic acid (4 μCi per incubation vial, specific activity 214 mCi/mmole) were purchased from the Radiochemical Centre, Amersham, Great Britain, the concentration of all precursors tested in the incubation medium was 10^{-3} M [2]. The incorporation time being 60 min, throughout the experiment the slices were treated with carbogen (95% O_2, 5% CO_2). At the end of incorporation the hippocampal slices were homogenized in 0.3 ml 0.1 N NaOH, from 0.2 ml of the homogenate the total proteins were precipitated using 0.5 ml ice-cold 12% trichloracetic acid. The pecipitate was washed twice with 6% trichloracetic acid, water, ethanol, dissolved in Hyamine hydroxide (New England Nuclear) and its radioactivity was determined in a liquid scintillation spectrometer (Intertechnique, Plaisir, France) using a dioxane containing scintillator. From the obtained values of radioactivity and protein content of the homogenate [5] the specific activity of proteins (d.p.m./mg protein) was calculated. For calculation of the incorporation rate IR (nmoles/mg protein/hour) the specific activity of proteins was corrected

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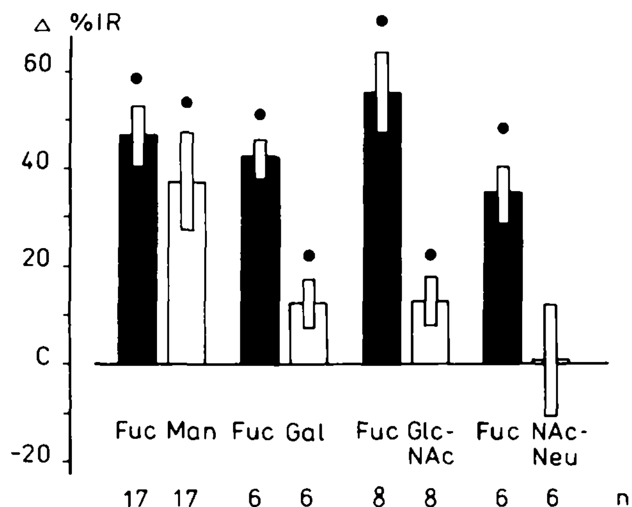

FIG. 1. Mean values of the percentage deviation of the incorporation rate IR of (^{3}H)-fucose and different sugars from control level (C) under the influence of 5×10^{-4} M dopamine. For comparison the influence of the transmitter substance on the incorporation of (^{3}H)-fucose and other sugars, respectively, parallely in slices of one hippocampus the IR of fucose and the sugars indicated was determined. In these experiments the IR (nmoles/mg protein/hour) of the different sugars were: (^{3}H)-fucose (Fuc) 0.41, (^{3}H)-mannose (Man) 0.50, (^{3}H)-galactose (Gal) 0.18, N-(^{3}H)-acetyl-glucosamin (GlcNAc) 0.20, N-acetyl-^{14}C-neuraminic acid (NAcNeu) 0.042. Statistical significance is based on Wilcoxon matched pairs signed rank test. ●$p<0.05$; n=number of animals.

by the amount of the precursor of the incubation medium using following equation:

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

RESULTS AND DISCUSSION

We compared in this investigation the changes in the incorporation of fucose into total proteins of hippocampal slices due to dopamine [4] with the influence of this transmitter on the incorporation of other sugars by determining their incorporation rates on slices from the same hippocampus. As shown in Fig. 1, the increase of incorporation of fucose and mannose due to dopamine did not quantitatively differ from each other. Both, fucose and mannose are incorporated as such from GDP-fucose and GDP-mannose, respectively. Whereas fucose becames coupled always in a terminal position of the glycan chain [1], mannose can be found also within the carbohydrate residue of the glycoproteins. The question arose, as to whether the effect of dopamine depends on the common kind of sugar activation by GDP-substitution, or on the position to which the sugar have to be coupled. The incorporation of galactose as well as of N-acetylglucosamine, which are incorporated as such from UDP-derivatives into glycoproteins [10] and mainly into a nonterminal position, is not influenced to the same degree by dopamine in comparison to that of fucose. N-acetyl neuraminic acid, a carbohydrate incorporated as such from the CMP-derivative [10] and only coupled in a terminal position of the glycan chain [1] showed no increase of incorporation by dopamine (Fig. 1). The results may suggest, that the influence of dopamine on the formation of glycoproteins seems not to be directed to the final completion of a glycan chain of glycoproteins, but merely to depend on the kind of nucleotides necessary for activation of sugars. The main influence could be obtained with GDP-sugars; the effect on the incorporation of UDP-sugars was much weaker, whereas the incorporation of the CMP-carbohydrate remained uninfluenced. Further investigations have to elucidate, whether these differences are due to a relatively specific regulatory effect of dopamine, or its second messenger systems within the neuron on the enzymatic reactions activating or transfering the GDP-sugars, or to the different availability of purine and pyrimidine nucleotides, respectively in the brain and resulting different rates of activation of the individual carbohydrates.

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