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Mechanisms of Dopamine Induced Changes in Hippocampal Glycoprotein Metabolism

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JORK, R., S. SCHMIDT, S. SCHULZECK, B. LÖSSNER AND H. MATTHIES. *Mechanisms of dopamine induced changes in hippocampal glycoprotein metabolism.* PHARMAC. BIOCHEM. BEHAV. 17(2) 203–207, 1982.—In rat hippocampal slices incubated in the presence of dopamine, a relatively strong correlation was observed between changes in the incorporation of ^{3}H-fucose into total proteins and the formation of GDP-^{3}H-fucose. However, in hippocampal homogenate the incorporation of ^{14}C-fucose from GDP-^{14}C-fucose was not stimulated by dopamine. In contrast, the incorporation of ^{3}H-fucose was stimulated by dopamine to a similar extent observed in hippocampal slices. Furthermore, in hippocampal slices dopamine did not increase the activity of fucosyltransferase. These results, together with our previous findings, suggest that the increased incorporation of fucose induced by dopamine in the hippocampal slices may be due to a receptor-mediated cAMP-dependent regulation, which controls the rate of fucosylation of acceptor-glycoproteins either at the level of fucose phosphorylation or of formation of GDP-fucose rather than the activity of fucosyltransferase.

Rat Hippocampus Dopamine Glycoprotein synthesis Fucose GDP-fucose Fucosyltransferase

ABUNDANT evidence supports the assumption that an increased formation of glycoproteins in the central nervous system plays a specific role in the changes of synaptic efficiency underlying memory processes [1, 9, 10, 13, 19, 22]. For example an increased incorporation of fucose into glycoproteins of rat hippocampus was found during as well as at different times after the acquisition of a foot-shock motivated brightness discrimination task. These changes in glycoprotein metabolism were different in the distinct hippocampal subfields and in certain glycoprotein fractions. Furthermore, dopamine or apomorphine stimulated the incorporation of ^{3}H-fucose into hippocampal glycoproteins both in vivo [15] as well as in vitro [14,18]. Thus, a linkage between the interaction of dopaminergic agonists with hippocampal receptor sites and alterations in glycoprotein synthesis can be assumed. It seems probable that these transmitter induced changes are mediated by a second messenger system. This assumption was confirmed since changes in glycoprotein synthesis similar to those induced by dopaminergic agonists were elicited by incubating hippocampal slices with dibutyryl-cyclic AMP [17,18].

In addition to the route of biosynthesis of GDP-L-fucose via GDP-D-mannose [7] a direct activation of fucose has been characterized [5]. This pathway involves the conversion of L-fucose to L-fucose-1-phosphate by a L-fucose-kinase [11] and the formation of GDP-L-fucose by a fucose-1-phosphate guanilyl transferase [12]. Finally, L-fucose is transferred to a terminal position of a carbohydrate chain by a fucosyltransferase [3,27]. Under conditions leading to an increased incorporation of fucose into hippocampal glycoproteins as described above, one or more steps of this pathway may be stimulated. In this paper we investigated whether the dopamine stimulated fucose incorporation is due to the alteration in the activity of the fucosyltransferase or to the stimulation of GDP-L-fucose synthesis.

METHOD

Incubation of Hippocampal Slices

For all experiments eight-week-old male Wistar rats from our own breeding stock were used. After the hippocampus was removed [21], 0.5 mm thick slices were prepared by cutting the structure perpendicularly to its longitudinal axis [26]. The slices were preincubated in 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) for 15 minutes. Throughout the experiment the medium was aerated with carbogen (95% O_2, 5% CO_2).

Assay of Fucosyltransferase Activity

The fucosyltransferase activity was assayed as previously described [27]. Hippocampal slices were incubated for various times with or without 5×10^{-4} M dopamine. After freezing on dry ice the slices were homogenized in 0.32 M sucrose. After a centrifugation at 11,000 g for 20 minutes, the resulting supernatant was further centrifuged at 125,000 g for 75 minutes. The pellet containing the microsomal fraction was then homogenized in 0.6 ml of a 0.2% Triton X-100 solution. After further centrifugation of the microsomal homogenate at 125,000 g for 75 minutes, the resulting supernatant was used for the enzyme assay. The reaction mixture

for the fucosyltransferase assay contained 50 μl of 0.1 M acetate buffer (pH 5.0), 100 μl of the enzyme extract (approximately 30 μg protein), 100 μl exogenous acceptor (0.5 mg sialic acid-free fetuin) and 50 μl GDP-(U-^{14}C)-fucose (190 nmole, specific activity 140 mCi/mmole, Radiochemical Centre Amersham, Great Britain). Incubation was carried out at 37°C for 30 minutes. The transfer reaction was stopped by addition of 1 ml of ice-cold 1% phosphotungstic acid in 12% trichloracetic acid. The resulting precipitate was washed twice both with 0.5% phosphotungstic acid in 6% trichloacetic acid and with ethanol-ether (1:1, v/v). Finally the pellet was dissolved in Hyamine hydroxide (New England Nuclear) and the radioactivity was measured in a liquid scintillation spectrometer using a dioxane containing scintillator. The fucosyltransferase reaction was shown to be linear over at least 60 minutes and over a range of 10 to 60 μg of solubilized microsomal protein.

Incorporation of Fucose into Total Proteins of Hippocampal Homogenate

The whole hippocampus was homogenized in 0.7 ml of ice-cold incubation medium used for slice experiments by ten up and down strokes. The incorporation of fucose was measured in a final volume of 0.5 ml containing 100 μl of hippocampal homogenate, 50 μl dopamine solution (final concentration 5×10^{-4} M) and 100 μl of radioactive labeled precursor. In some experiments 1 nmole GDP-(U-^{14}C)-fucose (specific activity 118 mCi/mmole) was added while in other experiments 1 nmole L-1-^{3}H-fucose (specific activity 500 mCi/mmole) was used. Both precursors were purchased from the Radiochemical Centre Amersham, Great Britain. After incubating for one hour the reaction was stopped by adding 1 ml of ice-cold 1% phosphotungstic acid in 12% trichloracetic acid. The resulting pellet was washed twice both with 0.5% phosphotungstic acid in 6% trichloracetic acid and with ethanol-ether (1:1, v/v) before dissolving in Hyamine hydroxide and counting for radioactivity.

Incorporation of Fucose into GDP-Fucose by Hippocampal Slices

Four hippocampal slices were incubated with or without a dopamine concentration of 5×10^{-4} M in a volume of 1 ml incubation medium. After 10 minutes 20 μCi L-1-^{3}H-fucose (specific activity 2 Ci/mmole) were added. After an incorporation time of one hour the tissue was homogenized in 0.5 ml of 70% ethanol containing about 4 nCi GDP-(U-^{14}C)-fucose (specific activity 118 mCi/mmole) as internal standard.

After centrifugation the resulting pellet was washed two times with each of the following solutions: 6% trichloracetic acid; ethanol-ether (1:1 v/v); and water and finally dissolved in hyamine hydroxide. The incorporation rate was expressed as relative specific activity (RSA); i.e., the ratio of the specific activity of proteins (d.p.m./mg protein) to the radioactivity of the free, nonincorporated ^{3}H-fucose. To the ethanol supernatant, 0.5 ml of 0.01 M ammonia formate was added, and the samples were extracted five times with a total amount of 15 ml of ether to remove the ethanol. Then, the samples were applied to a DOWEX-column (type 1×8, 200–400 mesh, formate form, 30×5 mm) [2]. GDP-^{3}H-fucose was eluted from the column using a discontinuous gradient of ammonium formate. For this the following ammonium formate solutions were subsequently added: 15 ml 0.01 M (containing 10 mM fucose); 15 ml 0.01 M; 10 ml 0.02 M; 9 ml 0.15 M; 6.5 ml 0.2 M and finally followed by 3.5 ml 0.5 N HCl. From each eluate, an aliquot was used for determining the radioactivity. The GDP-fucose is eluted by 0.5 N HCl. Approximately 80% of the internal standard (GDP-^{14}C-fucose) was removed in the 0.5 N HCl. The value of the GDP-^{3}H-fucose was corrected by this amount. This corrected value was further related to the radioactivity of the free, unconverted ^{3}H-fucose as determined from the sum of the 0.01 M and 0.02 M eluate.

TABLE 1

CHANGES IN THE ACTIVITY OF FUCOSYLTRANSFERASE ACTIVITY OF HIPPOCAMPAL SLICES INCUBATED DIFFERENT TIMES IN THE PRESENCE OF 5×10^{-4} M DOPAMINE

Incubation time of slices (min)	2.5	10	70
d.p.m. ^{14}C-fucose transferred by the enzyme of control slices	2522 ± 7	2258 ± 20	2024* ± 91
d.p.m. ^{14}C-fucose transferred by the enzyme of dopamine treated slices	2627 ± 10	1652 ± 24	1400* ± 45
Percent of controls	+4.2	−26.8	−30.9

To investigate changes in the activity of fucosyltransferase the hippocampal structure of 10 rats was prepared, sliced and pooled. One half of the slices was incubated in 3 ml of control medium, the other one in the presence of dopamine for the indicated times. Thereafter the activity of the fucosyltransferase of the slices of both groups was determined by incubating the solubilized enzyme for 30 minutes under conditions described above. Values represent the mean ± S.E.M. of three determinations of ^{14}C-fucose transferred from GDP-^{14}C-fucose to the acceptor protein under the experimental conditions described.

*Mean value ± S.E.M. of three measurements in three independent experiments.

RESULTS AND DISCUSSION

As described previously the incorporation of fucose into hippocampal glycoproteins was increased when slices of this brain structure were incubated in the presence of 5×10^{-4} M dopamine [14, 15, 18]. One can assume that this effect is due to a receptor-mediated cAMP-dependent regulation, which controls the intracellular conversion of the sugar [17,18]. Testing dopamine congeners on dopamine-sensitive adenylate cyclase (D_1 sites) in rat striatum or limbic tissue [24] EC_{50} values were found, which were in accordance with those concentrations of dopamine (10^{-6} to 10^{-5} M), which are thought to be present in the synaptic cleft during the discharge of dopaminergic vesicles [24]. However in phosphorylation experiments using mammalian superior cervical sympathetic ganglion [20], in electrophysiological investigations in hippocampal slices [8] as well as in studies of cyclic AMP-generating system of the hippocampal structure [25] concentrations of about 10^{-4} M dopamine or other transmitter substances were needed to elicit appropriate effects. Also in our studies such a concentration of dopamine was necessary to induce changes in the hippocampal glycoprotein formation.

It was the aim of this study to find out whether the dopamine stimulated fucose incorporation [14, 15, 18] is due to alterations in the activity of the fucosyltransferase or the stimulation of GDP-fucose synthesis. The activity of the fucosyltransferase relative to controls was estimated in the hippocampal slices after incubation for different times in the presence of dopamine (5×10^{-4} M). As shown in Table 1 the activity of this enzyme was unchanged after 2.5 minutes. However, after 10 and 70 minutes dopamine decreased the transfer of ^{14}C-fucose from GDP-^{14}C-fucose. In contrast to the decreased activity of this enzyme the incorporation rate of ^{3}H-fucose measured parallely in hippocampal slices from the same animals was increased by 43% after 70 minutes in the presence of dopamine as described earlier [14,18]. This apparent discrepancy may account for the inhibition of fucose transfer due to the formation of a complex consisting of desialyl-fetuin-enzyme-GDP [3]. Even if this inhibition should not occur with physiological acceptor-glycoproteins, the observed increase of fucose incorporation by dopamine seems not to be due to an increased activity of fucosyltransferase in our preparation. In order to rule out possible limitations in the responsiveness of the fucosyltransferase due either to the preparation procedure or to the incubation conditions in the assay system a further experiment was performed. In this experiment the influence of dopamine on the incorporation of ^{3}H-fucose as well as ^{14}C-fucose (from GDP-^{14}C-fucose) was investigated using a hippocampal homogenate. As demonstrated in Fig. 1 the incorporation of ^{3}H-fucose into total proteins was increased by dopamine (5×10^{-4} M). In contrast, the incorporation of ^{14}C-fucose from GDP-fucose was unchanged. These results support the assumption that the transfer of fucose by the fucosyltransferase reaction is not influenced by dopamine. Also, in other investigations [4] where an increased fucose incorpo-

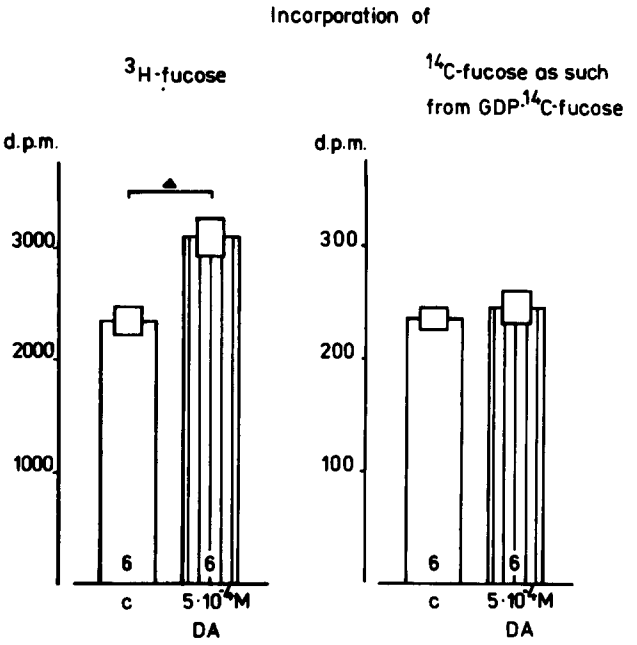

FIG. 1. Influence of dopamine on the incorporation of ^{3}H-fucose and ^{14}C-fucose from GDP-^{14}C-fucose into total proteins of hippocampal homogenate. To separate aliquots of a homogenate of the same hippocampal structure ^{3}H-fucose or GDP-^{14}C-fucose was added and incubated for one hour under control conditions (C) and in the presence of dopamine (DA, 5×10^{-4} M). Thereafter the incorporation of the different labeled precursors into the total proteins of the homogenate was determined. The present data are means of 6 animals in each case ±S.E.M. Statistical significance is based on the Wilcoxon matched pairs signed rank test (▲ $p<0.05$).

TABLE 2

FUCOSE INCORPORATION INTO TOTAL PROTEINS AND LABELLING OF GDP-FUCOSE IN CONTROL AND DOPAMINE TREATED SLICES

Control slices					Dopamine (5×10^{-4} M)				
A	B	C	A/C	B/C	A	B	C	A/C	B/C
5.61	21.97	3.79	1.48	5.79	11.40	38.58	3.90	2.92 (+97.3%)	9.89 (+70.8%)
7.76	54.06	3.95	1.96	13.69	9.73	64.89	3.67	2.65 (+35.2%)	17.68 (+29.1%)
8.60	66.06	4.21	2.04	15.69	7.15	52.71	3.03	2.36 (+35.2%)	17.40 (+10.9%)
7.52	40.49	4.54	1.66	8.92	8.49	50.56	3.94	2.15 (+29.5%)	12.85 (+44.1%)
6.07	51.21	4.52	1.34	11.32	7.52	47.80	4.06	1.85 (+38.1%)	11.77 (+3.9%)
5.51	39.64	6.41	0.86	6.18	11.35	79.55	6.99	1.62 (+88.3%)	11.38 (+84.1%)
5.43	56.34	5.85	0.93	9.63	7.00	60.94	4.86	1.44 (+54.5%)	12.54 (+30.2%)
5.86	46.28	5.10	1.15	9.07	11.35	90.24	4.79	2.37 (+106.1%)	18.84 (+107.7%)
9.05	105.47	7.09	1.28	14.87	12.98	155.70	6.35	2.04 (+59.3%)	24.53 (+64.9%)
2.55	71.53	7.26	0.35	9.85	3.60	115.65	7.17	0.50 (+42.9%)	16.13 (+63.8%)
3.74	104.40	6.88	0.54	15.17	4.19	129.08	6.83	0.61 (+12.9%)	18.90 (+24.6%)

A=Specific activity of proteins (d.p.m. $\times10^{3}$ ± mg protein).
B=GDP-^{3}H-fucose (d.p.m. $\times10^{3}$/mg protein).
C=Unconverted ^{3}H-fucose (d.p.m. $\times10^{5}$).
A/C=Relative specific activity of proteins (RSA).
B/C=Relative activity of GDP-^{3}H-fucose.
In parentheses: percentage deviation to appropriate values of control slices.

ration was observed in the synaptic membrane fraction of the visual cortex, no increase in the activity of fucosyltransferase was detected in the same brain region. For this reason, the authors [4] favor the assumption that the fucosylation reaction is not the step which regulates the observed alterations in the glycoprotein metabolism. Thus, it seems probable that the dopamine stimulated fucose incorporation is mediated by one of the other steps of the pathway of fucose conversion. Theoretically an increased formation of GDP-fucose could be responsible for the alterations in sugar incorporation too. To investigate this question, intracellularly formed GDP-^{3}H-fucose was separated by a DOWEX-column chromatography from the supernatant of hippocampal slices incubated in the presence of 5×10^{-4} M dopamine which is known to alter the rate of fucose incorporation into total hippocampal proteins [14,18]. Parallel to the estimation of the rate of GDP-^{3}H-fucose formation from ^{3}H-fucose, the incorporation of this sugar into total proteins of the same slices was determined (Table 2). A relatively strong correlation was observed between the dopamine-induced labelling of GDP-fucose and the dopamine-stimulated sugar incorporation into total proteins (Fig. 2). Therefore, it seems probable that alterations in the incorporation of fucose into total proteins of hippocampal slices incubated in the presence of dopaminergic agonists [14, 15, 18] are due to changes in the rate of the formation of nucleotide-sugar derivatives [16]. But this increase in nucleotide-sugar derivative formation could also be a consequence of an enhanced synthesis of fucose-phosphate, which should be investigated in further experiments.

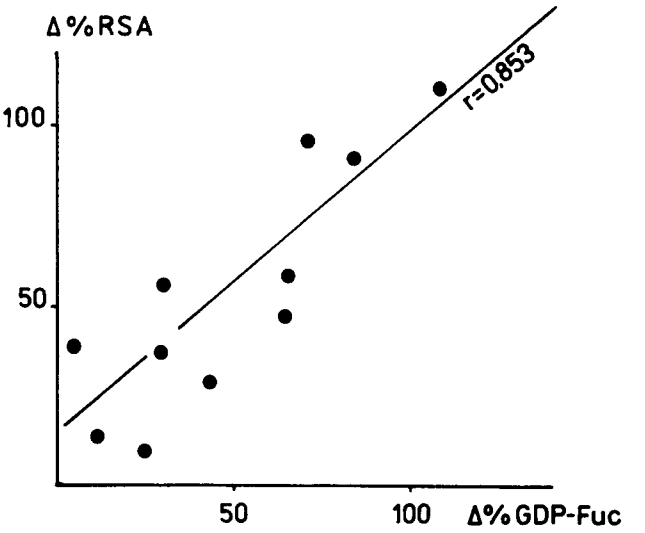

FIG. 2. Comparison of the percentage deviation of the labeling of GDP-fucose and values of RSA (relative specific activity of proteins) between slices incubated under control conditions and in the presence of 5×10^{-4} M dopamine. For this from the tissue of the hippocampal slices both the specific activity of proteins and the value of the radioactivity of formed GDP-^{3}H-fucose were determined. Both values were corrected by the radioactivity of the unconverted precursor separated from GDP-fucose by the DOWEX-column. Thereafter the percentage deviation of the corrected values between controls and dopamine treated slices was calculated (for values see Table 2).

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