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Impairment of Glycoprotein Fucosylation in Rat Hippocampus and the Consequences on Memory Formation

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JORK, R., G. GRECKSCH AND H. MATTHIES. *Impairment of glycoprotein fucosylation in rat hippocampus and the consequences on memory formation.* PHARMACOL BIOCHEM BEHAV 25(6) 1137–1144, 1986.—The intraventricular injection of 2-deoxy-D-galactose led to a dose- and time-dependent decrease in the fucosylation of hippocampal glycoproteins in rats whereas the incorporation of ^{3}H-N-acetyl-glucosamine was not influenced. This effect is not related to an interference with fucose activating or transferring enzymes but can be abolished by an application of D-galactose. Thus, it seems likely that also in brain tisuse a deoxy-galactose induced decrease in the fucosylation is due to a hindering of a glycosidic linkage of fucose to the deoxy-sugar incorporated into glycoprotein chains. As a consequence of an intrahippocampal injection of the deoxy-sugar the retention performance of the animals in a foot-shock motivated brightness discrimination task was considerably impaired. But deoxy-galactose is effective only when administered before and immediately after training whereas either a pre- or a post-training injection did not influence the retention performance of the rats. Thus, an effective metabolic inhibition of the glycoprotein completion by the deoxy-sugar starting at the time of training seems to be crucial to interfere with such morphofunctional alterations in the neuronal network underlying the formation of a memory trace.

Rat Hippocampus Glycoproteins Fucose 2-Deoxy-D-galactose Memory

DURING the last years the enormous variety of functions realized by glycoproteins as carriers of biological information began to be understood. Thus, glycoproteins are involved in the stabilization of certain protein conformations and in processes of the interaction with extracellular or intracellular molecules. Furthermore, cellular differentiation, cell adhesion and intercellular recognition are determined by glycoproteins to a high degree [1, 2, 16, 29, 30].

There is evidence that glycoproteins as prominent constituents of biological membrane structures are involved in processes underlying the modulation of synaptic connectivity during the formation of a memory trace. For that, changes in the glycoprotein composition of synaptic membranes and/or alterations in the carbohydrate portion of certain glycans has been assumed [1, 5, 12, 16, 17, 21, 24]. Thus, previous studies from several laboratories have demonstrated a variety of alterations in brain fucose metabolism using different learning paradigms [4, 5, 12, 14, 17–21, 24, 25, 35]. In appropriate investigations on a foot-shock motivated brightness discrimination task posttrial increases in the incorporation of ^{3}H-fucose into hippocampal glycoproteins were observed in our laboratory by gelelectrophoretic [20], lectin-binding [21] and histoautoradiographic [19] studies. Such alterations in the fucosylation of brain glycoproteins seem to be associated with an engram formation as they did not occur in trained, but rendered apparently amnestic, animals [24].

Moreover, due to a stimulation of hippocampal dopamine receptor sites both an increase in the incorporation of ^{3}H-fucose into glycoproteins mediated by receptor coupled events [9–12] and an improvement of the retention performance of rats in a discrimination reaction [7] were observed.

Finally, the improved retention of a learned behavior in rats induced by an intracerebral injection of fucose itself [37] points to the assumed link between alterations in the fucosylation of brain glycoproteins and mechanisms involved in memory storage. To characterize such a context in more detail it would be of interest to alter the composition of glycoproteins in order to inhibit their fucosylation and to investigate the consequence of such a modification on processes underlying neuronal plasticity. Whereas changes in protein glycosylation can be induced by the action of protein synthesis inhibitors [36], antibiotics [27] and by more recently characterized glycosylation inhibitors [28], due to an application of 2-deoxy-D-galactose in rats the incorporation of ^{3}H-fucose into acid-precipitable fractions of serum, liver and hepatoma was found to be inhibited partially but rather specifically [3]. Since fucose is coupled in some glycoproteins to galactose by a Fuc (1→2)Gal glycosidic linkage exclusively [2,29], the effect of 2-deoxy-D-galactose seems to be related to the incorporation of the deoxy-sugar into glycoprotein chains [3, 32–34], replacing galactose and hindering fucose to occupy its C-2 position [3]. Therefore, 2-deoxy-D-galactose could be a useful tool to study effects of

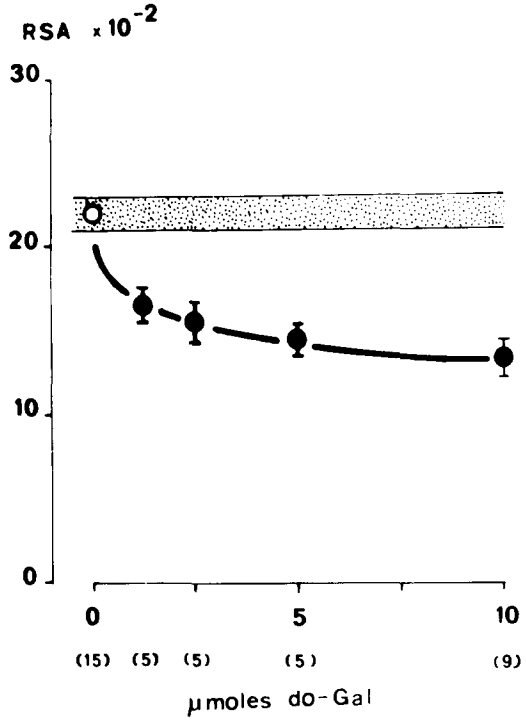

FIG. 1. Influence of 2-deoxy-D-galactose (do-Gal) on the incorporation of ^{3}H-fucose into hippocampal glycoproteins. Indicated doses of do-Gal were injected intraventricularly as 10 µl one hour prior to an application of 50 µCi ^{3}H-fucose, incorporation time being 1 hour. Mean values (±S.E.M.) of the relative specific activity of proteins (RSA) obtained under control conditions (white symbol) and after an injection of different doses of do-Gal (black symbols) are shown. In parentheses: number of animals. Differences between the control value and the experimental ones were found to be statistically significant according to the U-test of Mann and Whitney ($p<0.01$).

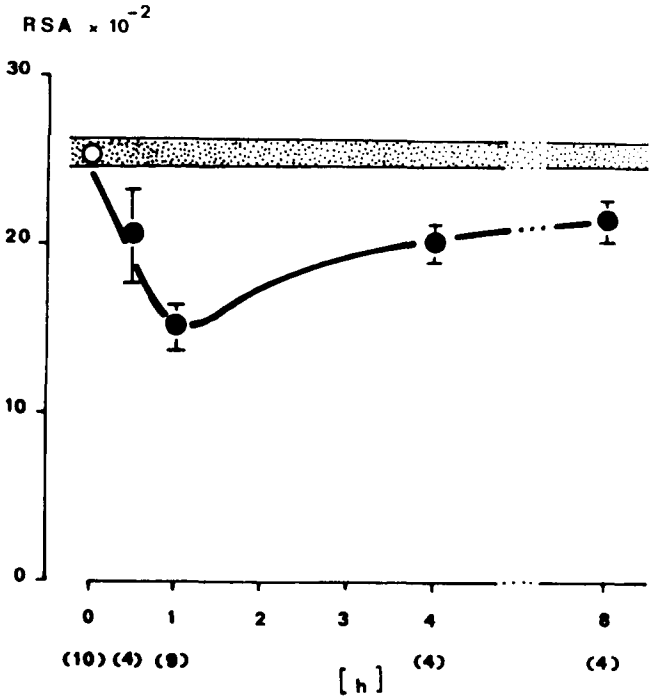

FIG. 2. Time dependency of the deoxy-galactose induced decrease in the fucosylation of hippocampal glycoproteins. An intraventricular injection of 5 µmoles of 2-deoxy-D-galactose was followed by a further application one hour later. At the times indicated 50 µCi of ^{3}H-fucose were applied, the incorporation time being 1 hour in each case. Time dependent changes in the relative specific activity of proteins (RSA, mean values±S.E.M.) obtained from treated rats (black symbols) are compared to the control level (white symbol ±S.E.M.). Differences between the control value and the experimental ones were found to be significant ($p<0.05$) at 1, 4 and 8 hours according to the U-test of Mann and Whitney.

an altered glycoprotein composition on neuronal mechanisms, presuming the deoxy-sugar acts in the same manner in the brain as in peripheral tissues.

Thus, in the present study the influence of 2-deoxy-D-galactose on the incorporation of ^{3}H-fucose into glycoproteins of the hippocampal structure was investigated. Moreover, the effect of the deoxy-sugar on glycoprotein fucosylation was compared to its influence on the incorporation of an other sugar, ^{3}H-N-acetyl-glucosamine. Furthermore, possible consequences of an altered glycoprotein composition of the hippocampus on processes of memory formation were studied by investigating the influence of an intrahippocampal injection of deoxy-galactose before and/or after a training experiment on a foot-shock motivated brightness discrimination task. The obtained effects of the deoxy-sugar were compared to the action of galactose in the same learning paradigm.

METHOD

Animals

In these experiments male Wistar rats from our own breeding stock weighing 210–240 g were used. The animals were housed under standard laboratory conditions in groups of ten rats per cage with food and water ad lib.

One week prior to the onset of learning experiments the animals were anesthetized with hexobarbital-urethane (100 resp. 600 mg/kg) and chronic microcannulas were implanted in each dorsal hippocampus (AP=3.1 mm, lateral=3.1 mm, vertical=3.1 mm) [31]. In biochemical investigations test substances and/or the radioactive labeled sugar were injected intraventricularly (AP=0.25 mm, lateral=1.6 mm, vertical=4.0 mm) [31].

Training Procedure

The training experiment was carried out using the learning task of a foot-shock motivated brightness discrimination as described earlier [7]. Initially, the animals were allowed to stay for 10 minutes in a semiautomatic Y-chamber for habituation. Thereafter, by application of 1 mA current to the grid floor, the animals escaped from the starting compartment. The animals had to learn to run in the illuminated alley of the Y-chamber. Runs into the dark alley were punished by footshocks and counted as errors. A run was considered positive if the animal ran into the illuminated goal alley immediately after connection of power. The light in the goal alley was switched off 20 seconds after the arrival of the rat.

After every three runs the direction of the illuminated alley was changed so as to avoid a position training. The mean time between two runs was about 60 seconds. The training session was completed after 31 trials. The retention performance was tested 24 hours after the training experi-

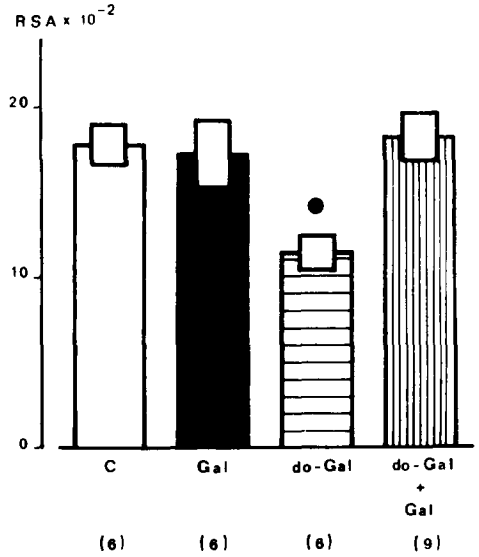

FIG. 3. Influences of D-galactose (Gal), 2-deoxy-D-galactose (do-Gal) and a mixture of both on the incorporation of fucose into hippocampal glycoproteins. Two and one hour prior to an intraventricular application of 50 μCi ^{3}H-fucose (incorporation time being 1 hour) 5 μmoles of each substance were injected at a volume of 10 μl. Mean values (±S.E.M.) of the relative specific activity of proteins (RSA) of the sugar pretreated animals are compared to such ones obtained from control rats (C). In parentheses: number of animals. ●$p<0.002$ according to the U-test of Mann and Whitney.

ment in an appropriate relearning session. The errors during training and relearning were used to calculate the saving scores according to the following equation:

$$\text{Percentage savings} = \frac{\text{training errors} - \text{relearning errors}}{\text{training errors}} \times 100.$$

Upon completion of the experiments the brains of the rats were microscopically checked for correct positioning of the cannulas. Only animals which showed an exact cannula positioning in the dorsal hippocampus were housed for evaluation.

To investigate the influence of 2-deoxy-D-galactose on the retention performance 1 μmole of this sugar analog was applied into both dorsal hippocampal structures using the schedules of injections as shown in Fig. 8.

The behavioral data from the experimental rats were compared to such ones which received an injection of physiological saline solution as the same volume and at appropriate times.

To specify an action of deoxy-galactose its influence on memory formation was compared to such one of D-galactose in equimolar doses using that paradigm of applications which revealed the most pronounced effect of the deoxy-sugar.

Biochemical Investigations

Fucose incorporation studies. One hour (in some experiments two and one hour) before an intraventricular application of 50 μCi ^{3}H-fucose (specific activity 2 Ci/mmole, Radiochemical Centre Amersham, Great Britain) dissolved in 10 μl of physiological saline solution, up to 10 μmoles of 2-deoxy-D-galactose at a volume of 10 μl were injected in the same way. Control rats received the same volume of physiological saline solution and the labeled sugar at appropriate times.

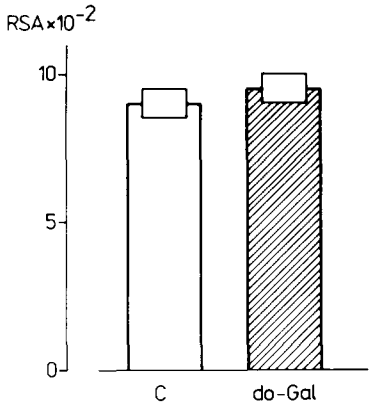

FIG. 4. Influence of 2-deoxy-D-galactose (do-Gal) on the incorporation of ^{3}H-N-acetyl-glucosamine into hippocampal glycoproteins. Five μmoles of do-Gal were injected intraventricularly as 10 μl two and one hour prior to an application of 30 μCi ^{3}H-N-acetyl-glucosamine, incorporation time being 1 hour. Mean values (±S.E.M.) of the relative specific activity of proteins (RSA) obtained under control conditions (white column) and after the injection of do-Gal (shaded column) are shown. In parentheses: number of animals.

After an incorporation time of the labeled precursor of one hour the animals were killed and the hippocampal structure of the injection site (right hemisphere) was dissected out. Thereafter the tissue was homogenized in 1.0 ml of 0.1 N NaOH. To 100 μl of this homogenate 500 μl of 12% trichloroacetic acid were added. The precipitated proteins were washed twice with 6% trichloroacetic acid, water and ethanol-ether (1:1, v:v). Finally, the pellet was dissolved in 0.5 ml Hyamine hydroxide and its radioactivity as well as that of 100 μl of the trichloroacetic acid soluble fraction was determined in a liquid scintillation spectrometer using a dioxane containing scintillator.

The obtained values of the radioactivity (d.p.m.) were corrected by the protein content of the tissue homogenate. Since fucose was found to be incorporated in glycoproteins exclusively and not to be converted to other monosaccharides the incorporation rate is expressed as the relative specific activity (RSA), i.e., the ratio of the specific activity of proteins to the radioactivity of the free, non-incorporated precursor of the soluble fraction. The influence of deoxy-galactose on the fucosylation of hippocampal glycoproteins was compared to its effect on the incorporation of an other sugar. Thus, one and two hours before an intraventricular injection of 30 μCi N-acetyl-D-(1-^{3}H)glucosamine (specific activity 2.84 Ci/mmole, Radiochemical Centre Amersham, Great Britain) 5 μmoles of 2-deoxy-D-galactose at a volume of 10 μl were applied in the same way. Corresponding controls received an injection of both physiological saline solution and the labeled sugar at appropriate times. After an incorporation time of the labeled precursor of one hour the incorporation rate of the sugar was determined using the biochemical procedure described above.

Assay of fucokinase activity. Fucokinase (E.C. 2.7.1.52) activity was estimated by measuring the transformation of ^{14}C-L-fucose to L-^{14}C-fucose-1-phosphate using a crude preparation of a soluble fraction of rat brain tissue. The enzyme assay was carried out according to the methods de-

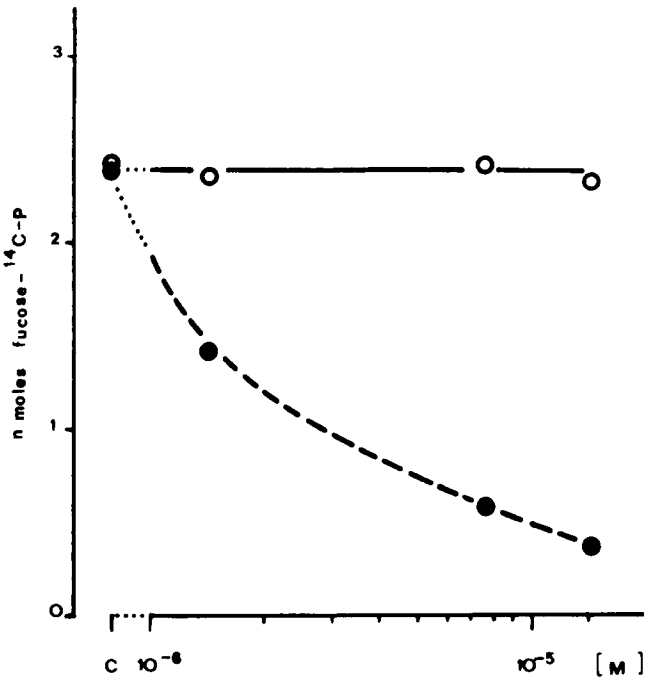

FIG. 5. Influence of different concentrations of 2-deoxy-D-galactose (○) and fucose (●) on the formation of fucose-1-^{14}C-phosphate from 1-^{14}C-fucose in the fucokinase assay mixture. Values are compared to such ones obtained for control conditions (C) and represent the mean of three independent determinations.

TABLE 1

FUCOSYLTRANSFERASE ACTIVITY IN PRESENCE OF 2-DEOXY-D-GALACTOSE

	d.p.m. ^{14}C-Fucose transfered by the enzyme per hour (mean values ± S.E.M. × 10^{-3})
Control	32.34 ± 0.72 (6)
5×10^{-7} M	34.06 ± 2.12 (3)
5×10^{-6} M	30.12 ± 1.39 (3)
5×10^{-5} M	30.34 ± 0.72 (3)
5×10^{-4} M	33.92 ± 0.86 (3)

2-Deoxy-D-galactose was added to the fucosyltransferase assay mixture to obtain the final concentration listed. Enzyme activity measurement was carried out as described in the Method section.
In parentheses: number of independent determinations.

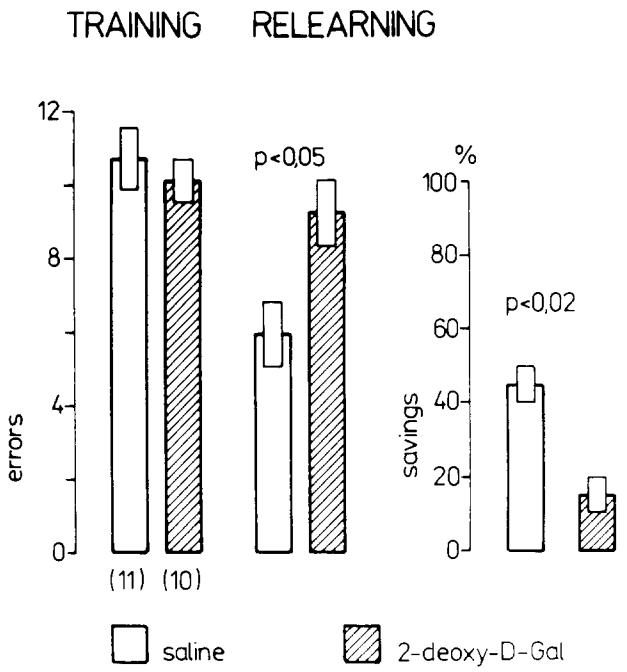

FIG. 6. Mean training and relearning errors (±S.E.M.) and the calculated percentage of savings from rats in a brightness discrimination task after one pre-training and two post-training intrahippocampal injections of 1 μl of physiological saline solution (white columns) and 1 μmole of 2-deoxy-D-galactose at the same volume (shaded columns). The applications were carried out according to the paradigm illustrated in Fig. 8A. In parentheses: number of animals. Significances according to the U-test of Mann and Whitney.

scribed in the literature for different tissues [8, 13, 23] and modified recently for brain tissues [12, 14, 21]. Briefly, brain tissue was homogenized in 9 volumes of 160 mM Tricin buffer, pH 8.5. The homogenate was centrifuged at 20,000 × g for 45 min. The obtained supernatant served as a crude enzyme source. Two hundred μl of this fraction were refilled to 320 μl incubation mixture containing 100 mM Tricin buffer, 4.8 mM ATP (Na^+ salt), 5.8 mM $MgSO_4$, 84 mM KF and 33 μM L-fucose-1-^{14}C (57 mCi/mmole, Radiochemical Centre Amersham, Great Britain, diluted with L-fucose to 10 mCi/mmole). After an incubation time of 45 min at 37°C the enzyme reaction was stopped by placing the mixture on a Dowex-column (type 2 × 8, 200–400 mesh, 30 × 5 mm, activated by 10 ml of a solution containing 190 g ammonium formate and 54 g of 85% formic acid per litre, pH 7.5). Thereafter the columns were washed with 10 ml of 10 mM ammonium formate solution, pH 7.5, and 0.5 ml of a solution containing 0.1 M HCl and 0.2 M KCl to remove the unconverted ^{14}C-fucose. Then L-^{14}C-fucose-1-phosphate was eluted by 2 ml of a solution containing 0.2 M HCl and 0.2 M KCl. To 1 ml of the obtained eluate 1 ml of water and 12.5 ml of dioxane containing scintillator (6 g PPO, 0.2 g POPOP, 100 g naphthalene, distilled dioxane at 1 litre) were added and counted in liquid scintillation spectrometer. Contaminations of the fucose-phosphate fraction by unconverted fucose were corrected by the value of radioactivity measured in this eluate after a complete reaction mixture was applied to the column without being incubated before. Blank values obtained by an incubation of the complete reaction mixture at 0°C for 45 min were finally subtracted from values of radioactivity representing fucose-phosphate. Due to the KF concentrations used the formation of GDP-fucose in the reaction mixture was less than 1%.

Fucokinase activity is expressed as nmoles L-^{14}C-fucose-1-phosphate formed per mg protein and one hour.

All estimations were carried out in triplicates. The protein content of the enzyme containing supernatant was determined using bovine serum albumin as standard [15].

Assay of fucosyltransferase activity. The fucosyltransferase (GDP-fucose: glycoprotein fucosyltransferase,

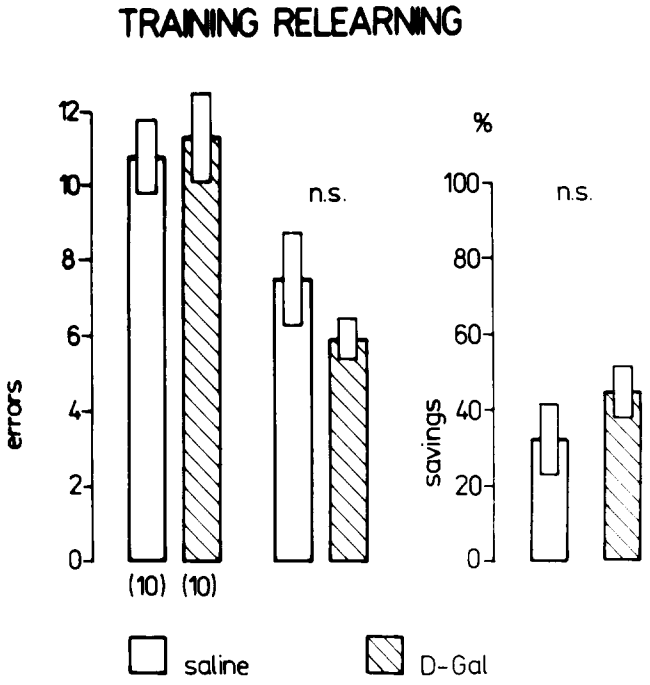

FIG. 7. Mean training and relearning errors (±S.E.M.) and the calculated percentage of savings from rats in a brightness discrimination task after intrahippocampal injections of 1 μl saline solution (white columns) and 1 μmole of D-galactose at the same volume (shaded columns) according to the application paradigm shown in Fig. 8A. In parentheses: number of animals.

E.C. 2.4.1.68) activity was estimated by measuring the transfer of L-fucose from GDP-L-fucose to an exogenous acceptor such as desialylated fetuin as previously described [38]. Brain tissue was homogenized in 9 volumes of 0.32 M sucrose. The homogenate was centrifuged at 11,000 × g for 20 min. The resulting supernatant was further centrifuged at 125,000 × g for 75 min. The obtained crude microsomal fraction was homogenized in 0.6 ml of a 0.2% Triton X-100 solution. After a further centrifugation of the microsomal homogenate at 125,000 × g for 75 min the resulting supernatant was used for the enzyme activity assay. The reaction mixture 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)-glucose (190 nmole, specific activity 140 mCi/mmole, Radiochemical Centre Amersham, Great Britain). Incubation was carried out at 37°C for 30 min. The fucose transfer reaction was stopped by the addition of 1 ml ice-cold 1% phosphotungstic acid in 12% trichloroacetic acid. The obtained precipitate was washed twice both with 0.5% phosphotungstic acid in 6% trichloroacetic acid and with ethanol-ether (1:1, v:v). Finally, the pellet was dissolved in Hyamine hydroxide 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 min and over a range of 10 to 60 μg of solubilized microsomal protein.

RESULTS

In a first series of experiments the influence of different doses of 2-deoxy-D-galactose on the incorporation of ^{3}H-fucose into hippocampal glycoproteins was investigated. As shown in Fig. 1 the intraventricular application of the deoxy-sugar reduced the fucose incorporation in a dose dependent manner. The maximal effect, a decrease in the sugar incorporation of 35–40%, occurred when 5 or 10 μmoles of 2-deoxy-D-galactose were injected. Investigating the fucose incorporation at different times after two injections of 5 μmoles of the deoxy-sugar a most pronounced decrease was seen after one hour in comparison to values obtained after 30 minutes, 4 hours or 8 hours (Fig. 2). Based on the assumption that the action of 2-deoxy-D-galactose is due to a replacement of galactose in appropriate glycoprotein chains, preventing a 1→2 glycoside linkage to fucose [3], the influence of an application of D-galactose on the deoxy-sugar induced reduction in fucose incorporation was investigated. Whereas after two intraventricular applications of 5 μmoles 2-deoxy-D-galactose the known decrease in the fucose incorporation was seen, after an equimolar injection of D-galactose or a mixture of D-galactose and the deoxy-sugar no changes in the sugar incorporation were found in comparison to control rats (Fig. 3). Moreover, in contrast to the decrease in the fucosylation of hippocampal glycoproteins induced by deoxy-galactose, no influence of the deoxy-sugar on the incorporation of N-acetyl-glucosamine was found (Fig. 4).

In order to rule out a possible interference of 2-deoxy-D-galactose with fucose activating or transferring enzyme systems the influence of the deoxy-sugar on fucokinase reaction and on the fucosyltransferase activity was investigated in vitro.

Whereas the addition of unlabeled fucose to the fucokinase assay mixture reduced the formation of labeled fucose-1-phosphate from ^{14}C-fucose to a high degree, 2-deoxy-D-galactose did not change the product formation in the same range of concentrations (Fig. 5). Also the activity of the fucosyltransferase was not influenced by deoxy-galactose even if concentrations up to 5×10^{-4} M were present (Table 1).

A possible influence of an altered glycoprotein composition of neuronal membranes on mechanisms underlying learning and long-term storage was tested using the behavioral task of a foot-shock motivated brightness discrimination task.

As demonstrated in Figs. 6 and 8A, animals that received intrahippocampal injections of 1 μmole 2-deoxy-D-galactose 30 minutes before, immediately and 1 hour after the training experiment showed a significant impairment of their retention performance compared to the corresponding controls.

In order to rule out a possible nonspecific influence of the relatively high concentrations of the sugar analog on the behavioral data, the action of D-galactose in equimolar doses was tested using the same experimental conditions and the same injection schedule. As shown in Fig. 7, due to three applications of 1 μmole D-galactose each no alterations in the retention performance of the treated rats were observed in comparison to the corresponding controls.

Moreover, to prove the question if a paradigm of three injections of 2-deoxy-D-galactose is necessary to induce the described effect on the retention performance (Fig. 6 and 8A) in a further series of experiments the influence of several pre- and/or post-training application(s) of the deoxy-sugar were tested.

Thus, as demonstrated in Fig. 8, 1 μmole of deoxy-galactose was applied into the hippocampal structure im-

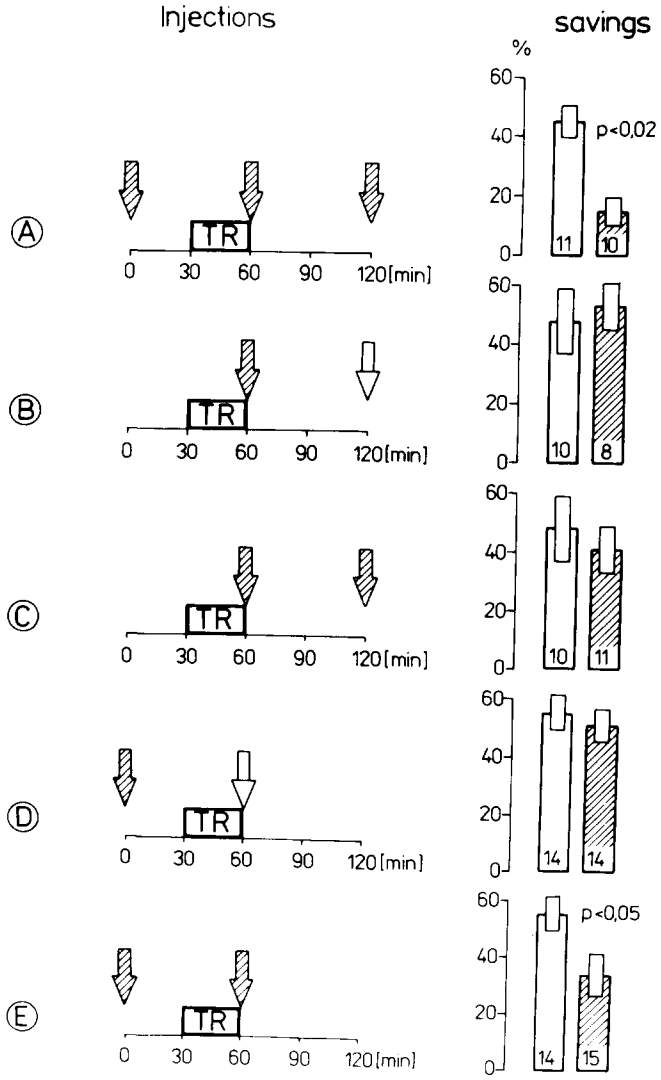

FIG. 8. Alterations in the retention performance of rats induced by different injection schedules of 2-deoxy-D-galactose. Left side: Paradigms of injections of 1 μmole deoxy-galactose (shaded arrows) and 1 μl saline solution (white arrows) at indicated times before and/or after the training session (TR) on a foot-shock motivated brightness discrimination task. Right side: Retention performance (expressed as percentage of savings) from deoxy-galactose treated rats (shaded columns) and corresponding controls (white columns). Control animals received at all arrow-indicated times an intrahippocampal injection of 1 μl saline solution. The results shown in B and C were obtained in one experiment involving one control and two experimental groups treated with 2-deoxy-D-galactose as indicated. The same is true for the data demonstrated in D and E.

mediately after the training experiment (B), in addition to that one hour later (C), 30 minutes before (D) and 30 minutes before as well as immediately after the training session (E), respectively. Due to one (Fig. 8B) or two (Fig. 8C) post-training injections of 1 μmole deoxy-galactose or a pre-training one (Fig. 8D) no significant alterations in the retention performance of the treated rats were found in comparison to corresponding controls.

But using an injection schedule with one pre-training and one post-training application of the deoxy-sugar (Fig. 8E) a significant impairment of the retention performance of the experimental rats was seen, although the effect was less pronounced in comparison to that observed when three applications of deoxy-galactose were done (Figs. 7 and 8A).

Moreover, in all cases where a pre-training injection of the deoxy-sugar was used (Fig. 8A, D, E) no differences in the mean value of errors made by experimental rats and control ones during the training session were observed (mean value of training errors of pre-training treated rats: 10.5, n=39; mean value of corresponding controls: 10.6, n=25).

DISCUSSION

Recent studies provided evidence for changes in the metabolism of brain glycoproteins during information processing and long-term storage [1, 5, 21].

Thus, in a variety of appropriate investigations increases in the incorporation of ^{3}H-fucose into glycoproteins realized by alterations in the activity of its activating or transferring enzymes were observed during, as well as after, acquisition of a new behavior [12, 20, 24, 25]. These alterations point to an involvement of certain glycoproteins in processes of such neuronal reconstructions underlying the formation of a memory trace. Especially intercellular relations are known to be determined to some extend by certain carbohydrate portions and/or specific oligosaccharide sequences of appropriate membrane glycoproteins [2]. Thus, using 2-deoxy-D-galactose we tried in the present study to modify the carbohydrate composition of some brain glycoproteins and to investigate the consequence of such an alteration on both glycoprotein fucosylation and memory formation.

2-Deoxy-D-hexoses are known to interfere with the glycoprotein synthesis by a different mode of action [3,27]. For 2-deoxy-D-galactose both its metabolism by enzymes of the Leloir pathway and its incorporation into glycoproteins of different tissues, including the brain, were described [32–34]. In peripheral tissues the incorporation of this deoxy-sugar in appropriate glycoproteins reduced a subsequent attachment of terminal fucose probably by hindering the formation of a 1→2 glycosidic linkage [3]. The dose- and time-dependent decrease in the incorporation of ^{3}H-fucose into hippocampal glycoproteins seen after an intraventricular application of 2-deoxy-D-galactose point to a similar mode of action of this deoxy-sugar in rat brain (Figs. 1 and 2). This assumption is supported by the findings that (1) fucose activating or transferring enzymes are not inhibited by this galactose analog (Fig. 5 and Table 1), (2) an injection of equimolar doses of D-galactose abolished the deoxy-sugar induced decrease in glycoprotein fucosylation (Fig. 3), (3) the incorporation of another sugar, i.e., N-acetylglucosamine, into carbohydrate chains of hippocampal glycoproteins is not affected by deoxy-galactose (Fig. 4). Although influences of 2-deoxy-D-galactose on some other pathways of the galactose and glycoconjugate metabolism cannot be ruled out completely it seems likely that its action is not related to a nonspecific inhibition of the glycoprotein syntheses in general, but could be partially due to a modification of some carbohydrate chains by the incorporation of this galactose analog replacing galactose and hindering a subsequent attachment of fucose to its C-2 position [3].

These assumed modifications of some hippocampal glycoproteins seem to be of rather functional importance. Thus, intrahippocampal injections of deoxy-galactose one time before and two times after the learning experiment on a foot-shock motivated brightness discrimination reaction re-

duced the retention performance of treated rats significantly (Figs. 6 and 8A). Interestingly enough, due to a more or less unspecific and indirect influence on glycoprotein synthesis by anisomycin and nucleotide trapping, the corresponding interference with the retention performance of trained animals was less pronounced [6,22]. Moreover, injections of equimolar doses of D-galactose at appropriate times did not influence the behavior of the rats in the relearning session in comparison to corresponding controls (Fig. 7).

Thus, nonspecific effects on the behavior of the animals due to an intrahippocampal application of relatively high doses of a sugar seem not to be the reason for the action of deoxy-galactose on the retention performance. Furthermore, the intrahippocampal injection of the deoxy-sugar revealed no alterations in the hippocampal EEG during, as well as after, the injection procedure (data not shown).

Testing other schedules of intrahippocampal deoxy-galactose injections on behavioral data it became evident that both a critical level of the sugar analog and a critical time period of its action are necessary to induce an impaired retention performance of trained rats by modifying hippocampal glycoproteins formed during, as well as shortly after, the acquisition of a new behavior (Fig. 8A and E). As known from incorporation studies and from histoautoradiographic investigations the period of training and the time after that is characterized by a pronounced increase in the formation of fucosylated glycoproteins crucial for memory formation [4, 5, 9, 12, 17, 19–21, 24]. Thus, an inhibition of these metabolic alterations during, as well as after, the time of training should interfere with the formation of a memory trace. This was true when deoxy-galactose was administered by one pre- and one (Fig. 8E) or two (Fig. 8A) post-training injections. Using these schedules of applications a substantial incorporation of the sugar analog into carbohydrate chains during the time period of an increased formation of glycoproteins can be assumed sufficient to interfere with memory formation. As demonstrated for brain tissue only 1% of the applied deoxy-galactose is transformed to the appropriate sugar nucleotide as a prerequisite step for its incorporation into glycoproteins [34]. Thus, by only one pre- (Fig. 8D) or by only one post-training (Fig. 8B) injection both the amount of modified glycoproteins and the time period of their availability seem not to be sufficient to interfere with the mechanisms underlying memory formation. Moreover, from the ineffectivity of two post-training applications (Fig. 8C) of the deoxy-sugar to impair the retention performance of the animals in the relearning session, a proactive effect of the drug as a reason for its action after one pre- and two post-training (Fig. 8A) or one pre- and one post-training (Fig. 8E) injection(s) can be ruled out. Thus, these results point to the necessity of a sufficient level of the sugar analog available during a period characterized by an increased formation of those glycoproteins involved in the formation of a memory trace in order to change the behavior of the animals in the relearning session. This could also be an explanation for the failure of one pre-training injection of deoxy-galactose to alter the behavior of the animals during the training as well as the relearning session (Figs. 6 and 8D).

In general, the obtained results provide further evidence for a crucial role of brain glycoproteins in such mechanisms underlying information processing and long-term storage.

Due to a modification of hippocampal glycoconjugates by an incorporation of deoxy-galactose and the subsequent partial prevention of their fucosylation it is tempting to speculate about a relative importance of those glycoproteins for the morphofunctional modifications involved in the formation of a memory trace. Thus, 2-deoxy-D-galactose could be a useful tool to select specific glycoproteins and to characterize their functions in processes of memory formation.

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