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New amino sugar analogues are incorporated at different rates into glycoproteins of mouse organs

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Abstract. Different radiolabelled N-acyl-derivatives of D-glucosamine were synthesized using D-glucosamine and the respective carbonic acid anhydride. Metabolism of these sugar analogues could be shown in vitro as well as in vivo. After the intraperitoneal administration of these radiolabelled N-acyl-D-glucosamines to mice, their rate of incorporation into glycoproteins of different organs was found to increase markedly with the length of the N-acyl side chain. Highest incorporation was measured in the whole intestine using N-pentanoyl-D-glucosamine as label.
Key words. Amino sugar analogues; liver; intestine; glycoproteins.

Since it became known that glycoconjugates play an important role in many biological processes, such as cell adhesion and antigenicity[1], different strategies have been established to elucidate the functional role of the oligosaccharide moiety of these complex compounds[2]. Hitherto, the most successful approach has been to use inhibitors of the glycosylation pathway, which are mostly inhibitors of glycosidases involved in the oligosaccharide maturation process[3]. We showed previously that the nonphysiological compound N-propanoyl neuraminic acid can be biosynthesized in the liver after administration of the nonphysiological amino sugar analogue, N-propanoyl-D-glucosamine[4]. In the work described in this paper we showed that N-propanoyl-D-glucosamine and related non-physiological glucosamine analogues can be incorporated into many organs of the mouse. However, they are incorporated at different rates, depending on the length of the modified N-acyl side chain.

Materials and methods

N-Acyl-D-glucosamines were synthesized according to a method described by Roseman and Ludowieg[5], using D-glucosamine and the respective carbonic acid anhydride. [1-^{14}C[-D-Glucosamine (60 mCi/mmol) was used for the synthesis of the corresponding radiolabelled N-acyl-D-glucosamines, and the reaction products were purified as described in an earlier study[6].

In order to demonstrate in vitro metabolism of the synthesized substances, a rat liver homogenate was prepared as described previously[7]. The assay system contained 200 µl rat liver homogenate, ATP (1.14 mmol/l), UTP (1.14 mmol/l), CTP (1.14 mmol/l), phosphoenolpyruvate (2.28 mmol/l) and pyruvate kinase (57 mU/µl), in a final volume of 350 µl. The reaction mixture was incubated for 30 min at 37 °C after addition of 100 nCi of the respective N-acyl-D-glucosamines (60 mCi/mmol), and the reaction was stopped by adding 500 µl ethanol. The precipitated proteins were centrifuged at 5000 × g for 3 min and the supernatants were analysed by descending paper chromatography[8]. Precursors were administered to male Balb C mice by intraperitoneal injection of 10 µCi (60 mCi/mmol). Under ether anaesthesia, the organs were removed 2 h later after perfusion with 15 ml 0.9% NaCl solution (w/v), weighed and transferred to chilled 0.9% NaCl solution containing 0.5 mmol/l $CaCl_2$. The organs were homogenized with an Ultraturrax, and cytosolic supernatants were obtained by centrifugation at 48,000 × g for 20 min. The pellet was solubilized with a Tris/HCl buffer containing 1% Triton X-100. Protein concentration was determined using the BCA assay according to Smith et al.[9]. Protein-bound radioactivity was measured by a modification of the procedure of Mans and Novelli[10]. For this purpose 200 µl samples were transferred to Whatman 3MM filter plates, precipitated three times with 10%, and treated twice with 5% trichloroacetic acid solution (w/v) for 30 min. The filter plates were washed three times with 50% ethanol solution (v/v), dried and radioactivity was measured by liquid scintillation counting[11]. Descending paper chromatography was carried out on Whatman 3MM paper using a solvent system containing n-propanol/water/1 mol/l sodium acetate, pH 5.0 (7:2:1, by vol.). The chromatograms were usually developed for 16 h, and for the determination of radioactivity, paper strips from chromatograms were measured by liquid scintillation counting[8].

Results and discussion

We synthesized five different radiolabelled N-acyl-D-glucosamines, usually with 95% purity, as precursors for the biosynthesis of glycoproteins. The reaction condi-

Table. Comparison of the metabolites after incubation of different N-acyl-[1-^{14}C]-D-glucosamines. 100 nCi of the respective radioactive labelled precursor were incubated in a 100,000 × g-supernatant of rat liver homogenate for 30 min and the percentage distribution of the metabolites was determined after analysis by descending paper chromatography.

Aminosugar [%]		Metabolites [%] Nucleotide-amino sugars	Aminosugar-phosphates	Neuraminic acid
N-Acetyl-[1-^{14}C]-D-glucosamine	10.3	17.2	72.5	-
N-Propanoyl-[1-^{14}C]-D-glucosamine	58.0	4.2	42.0	1.2
N-Butanoyl-[1-^{14}C]-D-glucosamine	38.9	-	61.0	-
N-Pentanoyl-[1-^{14}C]-D-glucosamine	80.6	-	19.4	-
N-Hexanoyl-[1-^{14}C]-D-glucosamine	37.2	16.7	46.0	ca.2
N-Crotonyl-[1-^{14}C]-D-glucosamine	80.9	3.6	2.1	ca.1

tions for the synthesis were established, and the structure of the products were confirmed by first using non-labelled substances.

Metabolization of the nonphysiologial precursors was shown by incubation with a rat liver homogenate. After a 30-minute incubation, the metabolites were analysed by descending paper chromatography. Four different types of metabolite were detected. By comparison of the R_f-values of the metabolites, and by enzymatic analysis, we showed that all the precursors investigated were phosphorylated.

The table shows a comparison of the metabolites found by descending paper chromatographic analysis. Compared to the other precursors investigated, N-hexanoyl-[1-^{14}C]-D-glucosamine was metabolized at the highest rate. Already 30 min after the start of incubation, 46% was phosphorylated and 16.7% converted to the respective nucleotide amino sugars. In the case of N-butanoyl- and N-pentanoyl-[1-^{14}C]-D-glucosamine, no conversion to the respective nucleotide sugars could be measured. In accordance with an earlier study, the formation of small amounts of N-propanoyl neuraminic acid was shown[10]. By in vivo administration of the nonphysiological sugar analogues to Balb C mice, we showed that all precursors were taken up and incorporated into the glycoconjugate fraction of different organs.

In all organs, except kidney and intestine, the highest uptake of radioactivity was found for the physiological N-acetyl-D-glucosamine (fig. 1). In kidney, N-hexanoyl-D-glucosamine showed the highest rate of incorporation, whereas in intestine N-pentanoyl-D-glucosamine showed the highest rate of incorporation.

In intestinal membrane glycoproteins, the rate of incorporation of amino sugar derivatives with 5 or 6 C-atoms in their N-acyl side chain was markedly higher than that of derivatives with fewer side chain C-atoms (fig. 2). This incorporation was even higher than that of the physiological precursor N-acetyl-[1-^{14}C]-D-glu-

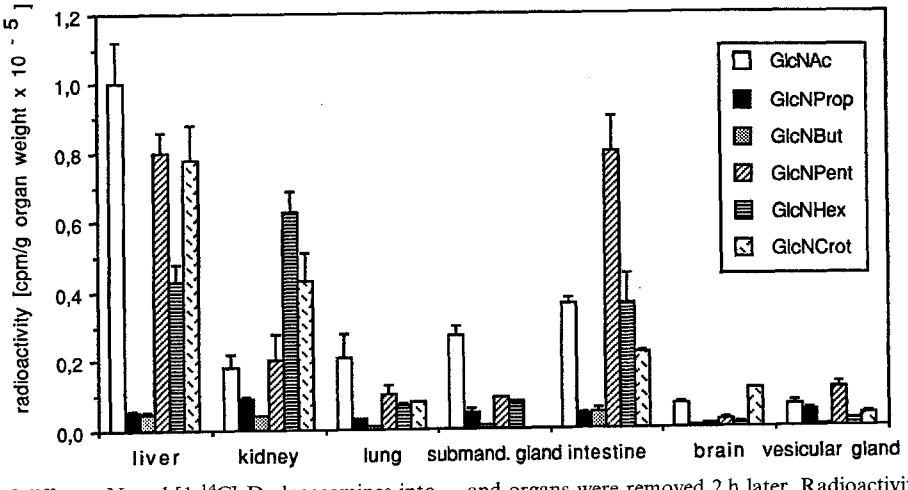

Figure 1. Uptake of different N-acyl-[1-^{14}C]-D-glucosamines into the crude membrane solubilisate of different mouse organs after intraperitoneal injection. Six groups (each of three mice) were labelled with the respective precursor. Each mouse received 5 µCi and organs were removed 2 h later. Radioactivity was determined in the supernatant (data not shown) and the crude membrance fraction. Values were determined in duplicate. Each column shows the mean for the respective organ of three mice ± SD.

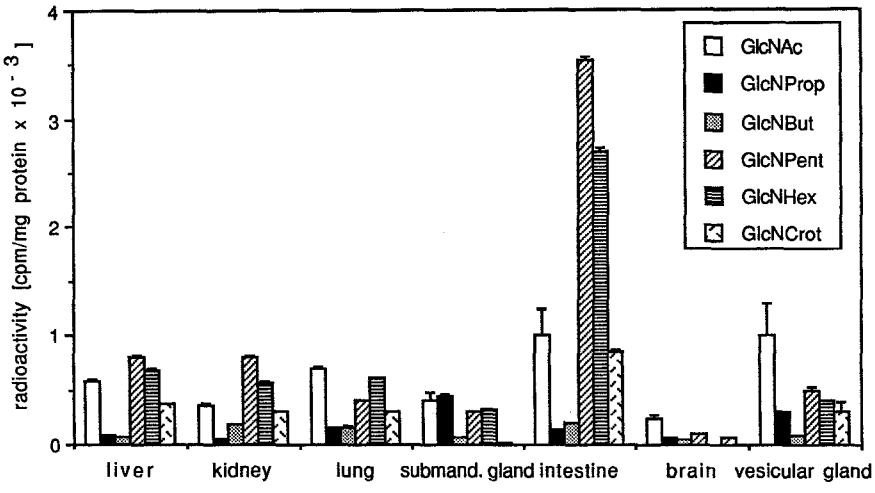

Figure 2. Incorporation of different N-acyl-[1-^{14}C]-D-glucosamines into the total fraction of membrane glycoproteins of different mouse organs after intraperitoneal injection. Six groups (each of three mice) were labelled with the respective precursor. Each mouse received 5 μCi and organs were removed 2 h later. Protein-bound radioactivity was determined in the supernatant (data not shown) and the crude membrane fraction. Values were determined in duplicate. Each column shows the mean for the respective organ of three mice ± SD.

cosamine. Similar data were obtained for liver and kidney.

There is as yet no satisfactory explanation for this high organ specificity linked to the length of the N-acyl side chain. The different values for the incorporation of the precursors into glycoproteins could possibly be explained by a difference in membrane permeability or intramembranous transport. Since we could show in a previous study[4] that N-propanoyl-D-glucosamine is converted to N-propanoyl neuraminic acid, we suppose that the N-acyl side chain could influence the activity of one of the respective sialyl transferases in different mouse organs. The activation to CMP-N-acyl neuraminic acid should not be influenced, because as Gross and Brossmer[12] were able to show, the relevant synthetase is not specific for the N-acyl group. We suggest that the use of these nonphysiological precursors for the in vivo modulation of glycoconjugates is a good tool for studying the biological role of a small component in one sugar of their oligosaccharide chains.

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