ELEVATED SIALYLTRANSFERASE ACTIVITY IN THE SERUM OF COLCHICINE TREATED RATS*

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SUMMARY: A strikingly elevated level of sialyltransferase activity was observed in the serum of rats treated with colchicine. In contrast, the level of other glycosyltransferases in the serum remained relatively unchanged with the exception of a smaller increase of fucosyltransferase activity. The increase of sialyltransferase activity in the serum was dependent upon the dose of colchicine and on the time following its administration. The possibility of presence of any activator or inhibitor of the sialyltransferase in serum was excluded by appropriate mixing experiments.

Secretion of lipoproteins and other serum proteins is greatly inhibited in colchicine-treated rats (1-3). This inhibitory effect appears to be at the site subsequent to the glycosylation of serum proteins (4). In eukaryotic cells, sensitivity of the cytoplasmic microtubules to colchicine is well established (5). Consequently, the effect of colchicine on a number of cellular processes is believed to be related to its primary interaction with the microtubules. A possibility of an interference at the level of the fusion of Golgi and plasma membranes has also been suggested to explain the effect of colchicine on the process of exocytosis. Disruption of exocytosis due to lack of membrane fusion may result from the action of colchicine on membrane binding sites involved in fusion. Golgi membranes enriched with glycosyltransferase play a role in the rapid renewal of plasma membranes (6), a process linked to exocytosis.

It is, therefore, possible that inhibition of exocytosis by colchicine is mediated through its effect on the glycosyltransferases in the

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rapidly renewing Golgi and other membranes. In course of our studies related to this problem, we have observed a strikingly elevated level of CMP-sialic acid: asialo-α_1-acid glycoprotein sialyltransferase activity in the serum of colchicine-treated rats.

METHODS

Adult Sprague-Dawley rats were exsanguinated under mild ether anaesthesia, blood was allowed to clot and serum was obtained by centrifugation at 2000 g for 15 min. Unless otherwise specified, sialyl- and galactosyltransferases were assayed in 10 µl serum (0.56-0.74 mg protein) in a total incubation volume of 50 µl. For sialyltransferase, the assay system contained MES-buffer (2(N-morpholino) ethane sulphonic acid), pH 6.8, 6.25 µmol; CMP- [4-^{14}C] sialic acid, 20 nmol, 0.01 µCi, 20,000 c.p.m. and 'desialized' α_1-acid glycoprotein, 250 µg as acceptor. For galactosyltransferase, the assay system contained MES-buffer, pH 6.8, 6.25 µmol; $MnCl_2$, 0.625 µmol; UDP- [U-^{14}C] galactose, 3 nmol, 0.025 µCi, 49,500 c.p.m. ATP 2 mM and 'desialized' and 'degalactosylated' α_1-acid glycoprotein, 250 µg as acceptor. N-acetylglucosaminyl- and N-acetylgalactosaminyltransferase activities were measured by previously described methods (7, 8) and fucosyltransferase activity was measured by the method of Chou et al. (9) using asialo-fetuin as exogenous acceptor. Incubation was for 1 hr at 37° C and the reaction was stopped with 1 ml of 10% (w/v) trichloroacetic acid/2% (w/v) phosphotungstic acid. The precipitate was filtered, washed, dried and counted for radioactivity to determine the pmol sugar incorporated into exogenous acceptor proteins (10). The protein was measured by using bovine serum albumin as standard (11). All enzyme assays were linear with time and with protein concentration in the incubation. In all enzyme assays the activity in absence of exogenous acceptor proteins was only 1 to 3% of that obtained in presence of exogenous acceptor.

Radioactive substrates were purchased from New England Nuclear Corp., (Dorval, Quebec) and purified human α_1-acid glycoprotein was a gift from the American Red Cross National Fractionation Center. Exogenous acceptors were prepared by the removal of appropriate sugars from α_1-acid glycoprotein by chemical methods (10).

Colchicine (from Sigma, St. Louis) was prepared freshly in 0.9% NaCl solution and was injected intraperitoneally into the rats.

RESULTS

Figure 1 shows the results of the assay of sialyltransferase in serum as a function of time of incubation (A) and of enzyme protein concentration (B). The massive increase of sialyltransferase activity in the serum of colchicine treated rats compared to saline treated controls

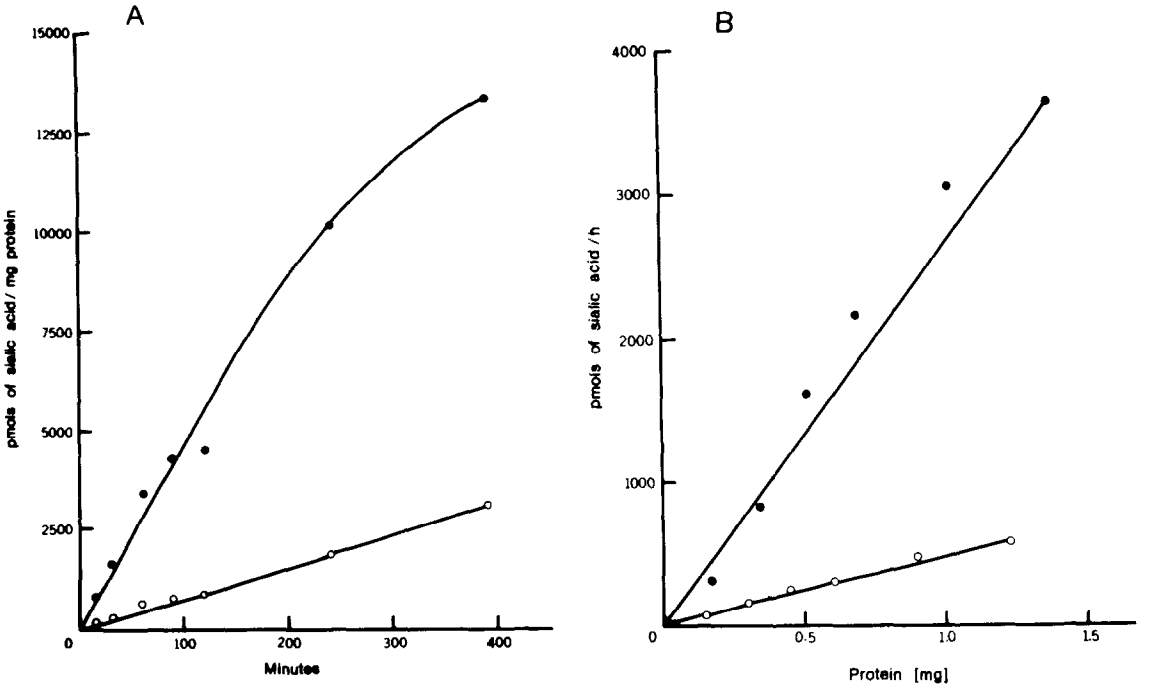

Fig. 1. Effect of colchicine on serum sialyltransferase. Control serum —o—; colchicine (0.5 mg per 100 g body wt)-treated (for 17 h) serum —●—. Dependence of enzyme activity on time of incubation (A) and on protein concentration (B).

is also clearly evident from the Figure. Figure 2 presents the results of sialyl- and galactosyltransferase assays in serum at different time intervals after colchicine (up to 24 hr) and also the effect of different doses of colchicine on the serum enzymes. Colchicine produces the maximal effect on the sialyltransferase with a dose of 0.25 mg/100 g body weight (A) and the enzyme activity continued to rise in serum up to the test limit of 24 hr (B). Figure 2 also shows that the effect of colchicine on the serum enzyme activity is restricted to a remarkable elevation of sialyltransferase, whereas the galactosyltransferase activity was only slightly affected. Experiments were also done to ascertain if the serum of control and colchicine-treated rats contained any inhibitory or stimulatory factor for the sialyltransferase. Results of the mixing ex-

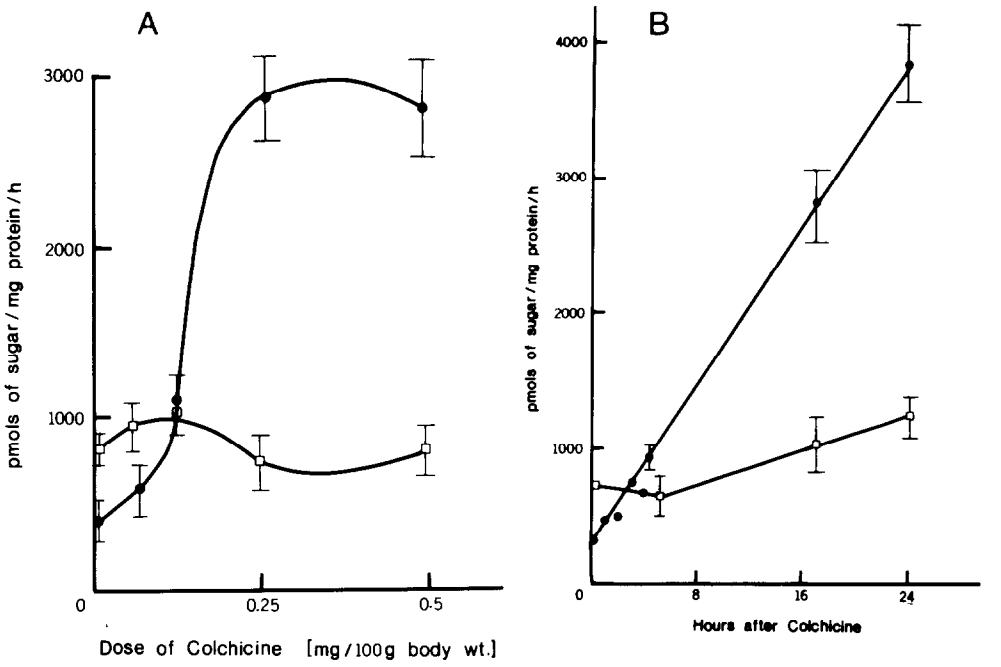

Fig. 2. Response of the serum sialyl- (—●—) and galactosyltransferase (—□—) to the dose of colchicine and on the time after colchicine treatment. For the dose response (A), the rats were killed 17 h after injections and for the time response experiment (B), 0.5 mg colchicine per 100 g body wt was injected into the rats. Each point is the mean of 4-6 rats and the vertical bars indicate S.E.M.

periments (Table 1) suggest that the serum from colchicine-treated rats did not activate the enzyme of the control rats, nor did the serum from the control rats have any inhibitory effect on the serum enzyme activity of colchicine-treated rats. Table 2 shows a two-fold increase of fucosyltransferase activity (statistically significant, $P < 0.01$), whereas three other glycosyltransferase activities remained unchanged by colchicine treatment.

DISCUSSION

Investigations have been carried out in recent years to relate the source, function and physiological significance of the occurrence of the

Table 1. Serum sialyltransferase activity: Effect of mixing serum from control and colchicine treated rats.

Colchicine (0.5 mg/100 g body wt) or saline was injected intraperitoneally into the rats. Blood was obtained after 17 h.

Experiment	Sialyltransferase activity (pmol/mg protein/h) Actual values	Sum of mixed assay	Difference
5 µl, control serum	199	1282	
5 µl, colchicine serum	1083		
5 µl, control + 5 µl colchicine serum	1391		+8%
10 µl, control serum	318	2467	
10 µl, colchicine serum	2149		
10 µl control + 10 µl colchicine serum	2346		-4.4%

soluble glycosyltransferases in serum and other tissue fluids (7, 10, 12-16).

Increased level of sialyl-, galactosyl- and N-acetylglucosaminyl-transferase activities in the serum of the patients with liver disease suggested that the serum enzymes are at least partly originated from liver (12, 14). Reports of elevated levels of galactosyl- and sialyl-transferase activity in the serum of patients or experimental animals afflicted with metastatic cancer and other related neoplastic diseases have appeared (12, 15, 16). These observations have focused on the significance of changes in serum enzyme activity as a reflection of abnormal cellular function in disease conditions. It is possible that the metastasizing tumor cells may produce and release more of these enzymes or increased turnover of cell surface in such conditions may have ac-

Table 2. Effect of colchicine treatment on serum glycosyltransferases.

Colchicine (0.25 mg/100 g body wt) or saline was injected intraperitoneally into the rats. Blood was obtained after 17 h. Values shown are mean from 3 to 6 rats.

Experiment	Glycosyltransferase Activity (pmol sugar/mg protein/h)				
	Sialyl-	Fucosyl-	Galactosyl-	N-acetylglucosaminyl-	N-acetylgalactosaminyl-
Control	253	2.24	996	160	8.2
Colchicine	1640	4.52	836	155	10.2

celerated the shedding of these cell surface enzymes into circulation. In this regard, our experiments with colchicine-treated rats offer an excellent model for correlating the cell-cycle and other membrane-related changes due to colchicine with the release or shedding of the surface and membrane-bound glycosyltransferase. Further significance is probably justified for the observation that colchicine treatment has a selective effect on the sialyltransferase and to a smaller extent on fucosyltransferase, enzymes which catalyse the transfer of the terminal sugar residues to glycoprotein acceptors. Sialic acid and fucose as terminal residues on the oligosaccharide chain may have special significance on the recognition mechanism for the membrane renewal and thereby on the process of exocytosis. Work is in progress to find out what specific tissue (or tissues) in the body is responsible for releasing large amounts of sialyltransferase into serum and also to ascertain what change in the cell due to colchicine produce such effect.

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