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Decreased Metabolism of Cerebrosides and Sulfatides in Rat Sciatic Nerve After Intraneural Injection of Colchicine

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Abstract: To obtain an understanding of the importance of the neuronal cytoskeleton in Schwann cell metabolism, an antimicrotubular agent (colchicine) was injected into the rat sciatic nerve 24 or 48 h before incubation of the nerve with labeled precursor: [^{35}S]sulfate, [^{14}C]galactose, or [^{3}H]-galactose. Colchicine inhibited the incorporation of ^{35}S radioactivity into sulfatides and, to a lesser extent, into proteins. With galactose as the radioactive precursor, synthesis of cerebrosides was reduced by colchicine injection, whereas incorporation of radioactivity into phosphatidylserine and phosphatidylcholine increased. Intraneural injection of lumicolchicine had no effect. The effects of colchicine on the metabolism of the Schwann cell are discussed in relation to its action on microtubules. **Key Words:** Colchicine—Lipids—Sulfatides—Cerebrosides—Rat sciatic nerve. **Souyri F. et al.** Decreased metabolism of cerebrosides and sulfatides in rat sciatic nerve after intraneural injection of colchicine. *J. Neurochem.* **51,** 599–604 (1988).

Colchicine, an antimicrotubular agent, is known as an antiinflammatory agent. In fact, it induces an extremely wide range of biological actions (Ellinger et al., 1983; Oda et al., 1983; Bennett et al., 1984), many of which are directly related to the binding of the drug to tubulin, i.e., disruption of the cytoskeleton (Banerjee and Battacharrya, 1979). In addition to its unexplained selective neurotoxicity on hippocampal granular neurons (Dasheiff and Ramirez, 1985), colchicine disturbs neuromediator release (O'Leary and Suszkiw, 1983) and axoplasmic transport (MacClure, 1972; Paulson and MacClure, 1975; Hanson and Edström, 1977, 1978). At even higher doses, impulse conduction is modified (Pellegrino et al., 1985; Dziegielewska et al., 1976). Although the action of locally applied colchicine on neurons has been demonstrated (Sloan et al., 1983), very little information is available on the effect of colchicine on glial and Schwann cells. In the CNS, injection of colchicine at doses sufficient to block axoplasmic transport has been reported to increase enzyme activity in glial cells and to increase the prominence of filaments (Hanson, 1972). Bizzozero et al. (1982) reported that in vitro colchicine inhibited the transport of vesicles containing glycoproteins from the Golgi apparatus to myelin in oligodendrocytes. Peripheral nerve is considerably simpler than the CNS and consists mainly of Schwann cells, myelin, and axonal fibers. Hugues et al. (1983) examined nerve ultrastructure and showed that Schwann cells were affected by colchicine. The purpose of the present study was to investigate the effect of colchicine on Schwann cell glycolipid synthesis.

MATERIALS AND METHODS

Materials

[^{35}S]Sulfate (as [^{35}S]sulfuric acid; 1;100 mCi/mmol) was from Amersham (Les Ulis, France). D-[^{14}C-U]Galactose (Gal; 210 mCi/mmol) and D-[6-^{3}H]Gal (28 Ci/mmol) were from C.E.A. (Saclay, France). The purity of colchicine (Calbiochem, La Jolla, CA, U.S.A.) was checked by TLC developed in a solvent system consisting of 5% methanol in chloroform with visualization under ultraviolet light (254 nm). All solvents were from Merck (Darmstadt, F.R.G.).

Animals

Each experiment was performed at least three times with four female Sprague–Dawley rats weighing between 150 and 250 g. Animals were anesthetized with sodium pentobarbital (Clin-Midy, Paris, France).

Schedule A. Both sciatic nerves of each animal were removed from the thigh and desheathed. The endoneurium was incubated with ^{35}S (20 μCi/nerve) with or without colchicine (100 μM) (see below).

Schedule B. Left sciatic nerves from each rat were injected with 1 μl of 10 mM colchicine (4 μg/injection) in

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Abbreviation used: Gal, galactose.

0.9% NaCl, whereas control right nerves received 1 μl of 0.9% NaCl. With the aid of an operating microscope, solutions were injected slowly into the endoneurium. Injections were made with a glass micropipette (tip diameter = 5 μm), obtained with an electrode puller (SRI, Ealing, France) held in a micromanipulator (Prior, France). The sharp tip of the micropipette penetrated close to the division into tibial and peroneal nerves and was directed toward the spinal cord. The other end of the micropipette was connected by means of a catheter to a peristaltic pump (Heape et al., 1986). A suture was placed in a nearby muscle to identify the site of injection; the wound was then closed with metallic clips. After recovery from anesthesia, no signs of limb paralysis were observed.

At 24 h after microinjections, sciatic nerves were removed, desheathed, and incubated for 3 h with either [^{35}S]-sulfate (20 μCi/nerve) or [^{14}C]Gal (5 μCi/nerve).

At 48 h after microinjections, sciatic nerves were removed, desheathed, and incubated for 3 h with [^{3}H]Gal (5 μCi/nerve).

Incubation, lipid extraction, and separation

For both schedule A and schedule B, incubations were performed in Krebs glucose-modified medium (Pleasure and Towfighi, 1972), with the pH adjusted to 7.35 at 37°C. After 3 h, incubation was stopped by replacing incubation medium with fresh, unlabeled medium. The endoneurium was homogenized with 8 volumes of distilled water/g of fresh tissue in a glass/glass tissue grinder, sonicated for 4 min in ice, and mixed with 15 volumes of chloroform/methanol (1:2 vol/vol). After centrifugation (5,000 rpm for 30 min), chloroform and water were added to the supernatant to obtain the Folch partition (Folch et al., 1957). Meanwhile, the protein pellet was dissolved in 0.1 *M* NaOH for counting of radioactivity and for protein content determination (Lowry et al., 1951). The supernatant biphasic system was centrifuged, and the lower phase (lipid extract) was washed twice with Folch's theoretical upper phase [chloroform/methanol/water (3:48:47 by volume)]. After evaporation of the lower phase under a flow of nitrogen, lipids were deposited on silicate plates (Merck, 60F254) and chromatographed using a mixture of methyl acetate/propanol/chloroform/methanol/0.9% KCl (25:25:25:10:9 by volume), as described by Vitiello and Zanetta (1978), for separation of each lipid class. Each spot, visualized by iodine vapor and identified using standards, was then scraped into Ready-Solv HP (Beckman) and counted for radioactivity with a Beckman scintillation counter. The distribution of the radioactivity between lipids was represented by histograms.

Incorporation of radioactivity into control and colchicine-treated nerves was compared using Student's *t* test.

RESULTS

Incorporation of [^{35}S]sulfate in the presence of colchicine

Effect of colchicine on in vitro incorporation of ^{35}S. After 3 h of incubation of nerves without added colchicine (control nerve), 8% of the radioactivity was found in the nerve homogenate, and this radioactivity was distributed between the water-soluble fraction (70%) and lipids and proteins (30%). Proteins were much more radiolabeled than lipids (Table 1). Chromatography showed that lipid radioactivity was present only in sulfatides.

When incubations were performed in the presence of colchicine (100 μ*M*), homogenate labeling represented 8.4% of the radioactivity and was not significantly different from values without colchicine. The radioactivity found in sulfatides and proteins was the same for nerves incubated with and without colchicine (see Table 1).

Effect of intraneurally injected colchicine on in vitro incorporation of ^{35}S. At 24 h after intraneural injection, 6.1% of the radioactivity was found in the nerve homogenate, and this radioactivity was distributed between the water-soluble fraction (77%) and proteins (22.5%), whereas little radioactivity (0.9%) was found in sulfatides (Table 1). The incorporation of label into proteins was much greater in control nerves after intraneural saline injection than in noninjected control nerves.

After colchicine injection, alterations of synthesis were evident (Table 1). Incorporation of [^{35}S]sulfate into proteins was decreased (62% as compared with control values of saline-injected nerves), as was incorporation of ^{35}S into sulfatides (57% as compared with controls). These differences were significant ($0.001 < p < 0.01$) and more marked than when colchicine was added to the incubation medium. Indeed, the colchicine/nerve contact time was 24 h "in situ" and 3 h "in vitro."

TABLE 1. *Incorporation of ^{35}S into proteins and sulfatides after incubation of sciatic nerves*

	Mean ± SEM dpm/mg of protein		
Nerve preparation	Homogenate	Proteins	Sulfatides
Without colchicine	356,683 ± 53,169	69,676 ± 6,886	5,923 ± 1,035
Plus colchicine (100 μ*M*)	369,793 ± 32,912	78,179 ± 10,798	5,428 ± 972
Control, injected with 1 μl of 0.9% NaCl 24 h before incubation	268,052 ± 26,624	60,135 ± 7,788	2,391 ± 536
Injected with colchicine (4 μg) 24 h before incubation	215,145 ± 17,356	37,138 ± 8,461[a]	1,363 ± 307[a]

Desheathed sciatic nerves were incubated for 3 h in modified Krebs medium with 20 μCi of [^{35}S]sulfate. After incubation, sciatic nerves were homogenized, and incorporation of radioactivity into protein and lipids was measured.

[a] $0.001 < p < 0.01$.

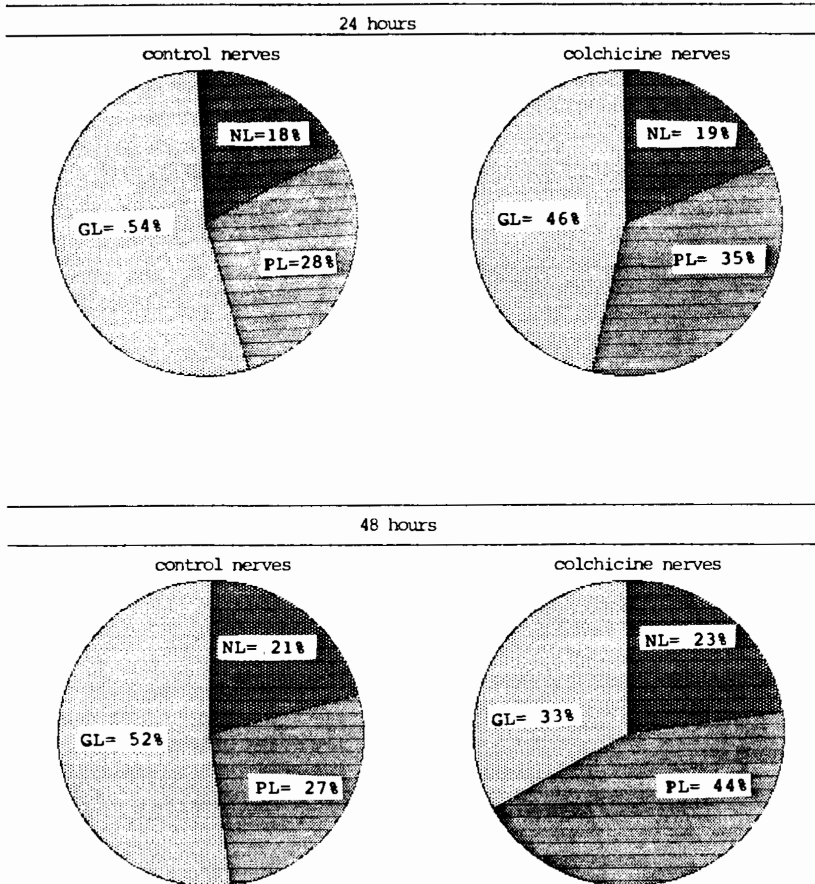

FIG. 1. Effect of colchicine on the labeling pattern of glycolipids (GL), phospholipids (PL), and neutral lipids (NL). Colchicine was injected intraneurally 24 (**top**) or 48 h (**bottom**) before incubation of sciatic nerves with [^{14}C]Gal or [^{3}H]Gal, respectively. Control nerves were injected with NaCl at the same time.

Incorporation of labeled galactose after intraneural injection of NaCl or colchicine

Effect of intraneurally injected colchicine 1 day before a 3-h incubation with [^{14}C]Gal. After intraneural injection of saline, 3.6% of the added radioactivity was found in the nerve homogenate. This radioactivity was distributed between the water-soluble fraction (89%), proteins (5%), and lipids (6%). In a comparison of incorporation of the two precursors (^{35}S and [^{14}C]Gal), the percentage of [^{14}C]Gal bound to proteins was much lower than that of ^{35}S (5 versus 22%), whereas the percentage of lipid labeling was greater (6% of [^{14}C]Gal bound to lipids versus 0.9% for ^{35}S). After separation of the different lipid classes (Fig. 1), ^{14}C radioactivity was found mainly (54%) in glycolipids, as expected: 90% in cerebrosides and 10% in sulfatides. Nevertheless, radioactivity was also found in the other lipids, such as neutral lipids and phospholipids. The most labeled phospholipids were phosphatidylcholine and phosphatidylserine (Fig. 2).

Comparison between saline- and colchicine-injected nerves showed an increase ($0.01 < p < 0.02$) in homogenate radioactivity after colchicine treatment. Nevertheless, when the percentage of incorporation is considered, colchicine did not significantly change the protein-bound radioactivity (Table 2). On the other hand, [^{14}C]Gal incorporation into lipids was depressed after colchicine injection as compared with control values. In addition, colchicine treatment resulted in lowering the glycolipid/phospholipid radioactivity ratio (Fig. 1) compared with controls (1.40 versus 2.11; $p < 0.001$). Incorporation of [^{14}C]Gal into the different lipids is shown in Fig. 2. Incorporation of [^{14}C]Gal into cerebrosides decreased significantly with colchicine treatment. This incorporation of radioactivity was due to Gal incorporation, as shown by hydrolysis of cerebrosides (Svennerholm, 1956). Sphingosine and free fatty acids accounted for 5.4% of the total radioactivity, whereas the aqueous fraction containing Gal accounted for 90 ± 0.5%. On the other hand, the incorporation of label into all phospholipids, especially phosphatidylserine and phosphatidylinositol, increased ($p < 0.05$; Fig. 2). After transmethylation of phospholipids (as described by Morrisson and Smith, 1964), fatty acids were not radioactive, whereas the aqueous extract containing glycerol was as labeled as phospholipids.

Effect of intraneurally injected saline or colchicine 48 h before incubation with [^{3}H]Gal. After intraneural injection of saline, 4.4% of the added radioactivity was found in the sciatic homogenate. This radioactivity was distributed among the water-soluble

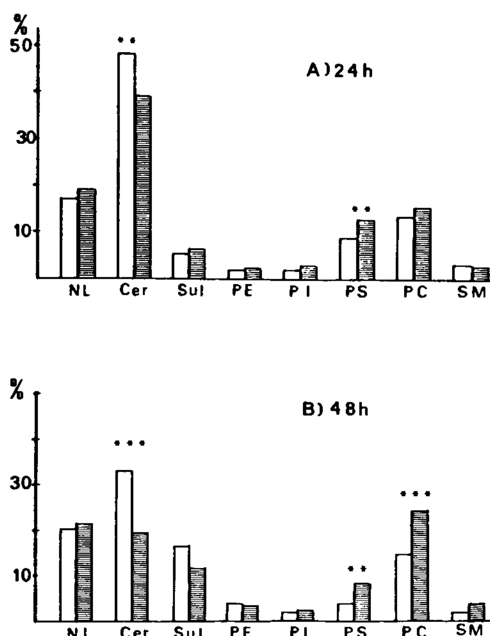

FIG. 2. Effect of colchicine on the labeling pattern of sciatic nerve lipids, shown as the percentage of radioactive Gal incorporated into lipids after incubation for 3 h. Colchicine (cross-hatched columns) was injected intraneurally 24 (**A**) or 48 h (**B**) before incubation of nerves with the precursor. Controls (open columns) were injected with 1 μl of 0.9% NaCl. Each experiment consisted of four rats, and at least three experiments were performed, so the total number of animals was 12. NL, neutral lipids plus monogalactosyldiacylglycerol; Cer, cerebrosides; Sul, sulfatides; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin. Values significantly different from controls are indicated: **$0.001 < p < 0.01$, ***$p < 0.001$.

fraction (87%), proteins (6.9%), and lipids (5.7%). Injecting saline at 24 or 48 h before incubation did not significantly alter the results. In addition, there was no difference of incorporation into several lipid classes; 21% of the ^{14}C label was associated with neutral lipids, 52% with glycolipids, and 27% with phospholipids (Fig. 1). The only difference observed between [^{3}H]Gal incubation and [^{14}C]Gal incubation was that after [^{3}H]Gal incubation (48 h), sulfatides were much more labeled (Fig. 2).

Comparison of incorporation of label into proteins (Table 2) 48 h after injection of saline or colchicine showed that colchicine did not significantly affect [^{3}H]Gal incorporation into glycoproteins. However, incorporation of [^{3}H]Gal into lipids was only 76% of control values (Table 2). Colchicine treatment resulted in a significant ($p < 0.001$) reduction of the glycolipid/phospholipid ratio, to 0.81 vs. 2.04 in controls (Fig. 1). This decrease resulted from the reduction of [^{3}H]Gal incorporation into both cerebrosides and sulfatides and from the increase of label incorporation into some phospholipids. Figure 2 shows that whereas incorporation of precursor into phosphatidylserine and phosphatidylcholine was increased ($p < 0.001$), incorporation into phosphatidylinositol and sphingomyelin was not affected.

DISCUSSION

The results presented here show that colchicine, when injected intraneurally at a dose of 4 μg, affects lipid metabolism of rat sciatic nerve Schwann cells in a highly significant manner. Colchicine is known to bind to tubulin and to impair slow and fast components of axonal transport. In the rat sciatic nerve, acetylcholinesterase transport has been reported to be blocked by 400 μg of intraneurally injected colchicine (Kreutzberg, 1969) and choline acetyltransferase transport to be inhibited by 200 μg of injected colchicine (Dziegielewska et al., 1976). At the same dose levels we used, tubulin transport has been shown to be specifically inhibited (Tashiro et al., 1984) and mitochondrial flow to be slowed down (Jeffrey et al., 1972). However, no one has examined colchicine's action on Schwann cells, i.e., whether axonal perturbations influence protein and lipid synthesis in these cells.

With [^{35}S]sulfate as the precursor, intraneurally injected colchicine appears to inhibit synthesis of sulfa-

TABLE 2. *Incorporation of labeled Gal into proteins and lipids after incubation of sciatic nerves*

Timing, agent injected	Mean ± SEM dpm/mg of protein		
	Homogenate	Proteins	Lipids
24 h before incubation with [^{14}C]Gal			
NaCl	398,466 ± 55,863	21,300 ± 4,751	23,831 ± 5,216[a]
Colchicine	422,040 ± 36,353	18,434 ± 2,622	15,667 ± 2,370[a]
48 h before incubation with [^{3}H]Gal			
NaCl	456,919 ± 36,405	31,326 ± 4,366	26,176 ± 2,223[b]
Colchicine	45,178 ± 28,564	30,331 ± 2,427	19,663 ± 1,381[b]

Desheathed sciatic nerves were incubated for 3 h in modified Krebs medium with 5 μCi of [^{14}C]Gal or [^{3}H]Gal. At the end of the incubation, radioactivity incorporated into protein and lipids was measured.
[a] $0.02 < p < 0.05$.
[b] $0.01 < p < 0.02$.

tides as well as the sulfation of proteins. The sulfation process takes place in the Golgi apparatus, so colchicine may be able to act directly on the functioning of the Golgi apparatus. Indeed, Townsend et al. (1984) showed that in the brain, colchicine inhibited the transport of Golgi-derived vesicles to the forming myelin membrane, but no direct inhibitory effect of colchicine on the Golgi apparatus was observed. Nevertheless, our results in sciatic nerve demonstrate a substantial difference of incorporation of ^{35}S into sulfatides. This could indicate an effect of colchicine either on cerebroside sulfotransferase or on a previous step in cerebroside synthesis.

The labeled Gal precursor was found to be incorporated into cerebrosides and sulfatides, but it was also degraded, and the radioactivity was incorporated into phospholipids and neutral lipids. After injection of colchicine, [^{14}C]Gal incorporation was similar to [^{3}H]Gal incorporation into proteins and into lipids. Protein synthesis was unchanged, as judged by [^{3}H]fucose incorporation (data not shown).

Thus, glycosylation of proteins in the Golgi did not seem to be affected by colchicine. Therefore, it appears unlikely that glycosylation of ceramides in the Golgi is the target of colchicine's action. It is possible that under our experimental conditions, colchicine inhibited ceramide galatosyl transferase. An action of colchicine on other enzymic activities has been shown in the liver (Mitranic et al., 1981) as well as in spinal cord (Ishida and Deguchi, 1984). The sharp rise in phosphatidylcholine and phosphatidylserine synthesis 1 or 2 days after colchicine injection may also be an indication of some nerve degeneration (Natarajan et al., 1982), although not strongly visible.

Because lumicolchicine and colchicine have similar properties with regard to binding to cellular membranes (Mizel and Wilson, 1972) but differ markedly in their binding capacity to microtubule protein (Dahlström et al., 1975), we performed another experiment with the same schedule but with lumicolchicine (4 μg intraneurally) instead of colchicine. We did not find any significant difference between saline- and lumicolchicine-injected nerves with respect to incorporation of radioactivity into proteins and lipids or into the different classes of lipids (data not shown). Thus, it appears that the effect of colchicine on lipid metabolism of the peripheral nerve may be due to its action on microtubules.

One question that remains is whether this action of colchicine due to an effect on axonal microtubules or to an effect on Schwann cell microtubules. In other words, are the results obtained here the consequence of inhibition of axonal transport, or are they the consequence of local inhibition of the Schwann cells? Experiments are now in progress to compare the effect of other drugs acting on the cytoskeleton, such as taxol and cytochalasin D.

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