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Altered metabolism of rat contralateral sciatic nerve after microinjection into the endoneurium of the ipsilateral sciatic nerve

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Microinjection of substances into the endoneurium of the sciatic nerve is a technique widely used. Because the contralateral nerve is often used as control nerve, we studied the incorporation of [^{14}C]galactose into myelin lipids in normal nerves, saline-injected nerves and their contralateral nerves. Results show that incorporation of radioactivity into contralateral nerve lipids is affected as compared with normal nerves. We conclude that the contralateral nerve of an injected nerve is not a good control.

Microinjection of various substances into nerve endoneurium is a technique widely used because of its numerous advantages: (i) no systemic effects with toxic substances, (ii) no transport across the blood–nerve barrier, (iii) only small quantities required and high labeling with radioactive precursors [5].

It is generally assumed that damage from the needle is minimal [2, 4]. Another assumption is that damage to the injected nerve (if there is any) is too small to provoke changes in the contralateral non-injected nerve; consequently many researchers use the contralateral nerve of the same experimental animal as control. Nevertheless, it is well known that injury to the ipsilateral nerve can have a transspinal effect [17]. Because our laboratory was interested in the study of peripheral nerve metabolism by means of endoneural injection, we decided to examine whether the contralateral nerve of injected nerves (contralateral nerve) has exactly the same metabolism as nerves from an intact animal, measuring the incorporation of [^{14}C]galactose into myelin galactolipids.

Two groups of 10 female Sprague–Dawley rats (Iffa Credo, France) weighing 150–200 g were used for this study. The first group did not receive any injection (control nerves). In the second group, rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (35 mg/kg b.wt.) and the right sciatic nerve was exposed by

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retracting the overlying muscle (taking care not to interfere with the nerve blood supply) and 1 μl of 0.9% saline was injected at 7.5 cm of the spinal cord. The volume was delivered slowly from a glass micropipette pulled with electrode puller (Ealing, France) and directed towards the spinal cord. Then, the wound edges were held together with a surgical clip. After 24 h, both groups were anesthetized and all sciatic nerves were removed at the site of the injection, desheathed, measured (length) and incubated 3 hours with 5 μCi of [^{14}C]U-D-galactose (210 mCi/mmol) from CEA (France) in a modified Kreb's glucose buffer [11] adjusted to pH 7.4. At the end of the incubation, nerves were washed twice by adding the ice-cold buffer, homogenized and lipids extracted by Folch's procedure [3]. An aliquot of the lipid extract was counted to obtain the total incorporation of [^{14}C]galactose into sciatic nerve lipids; an aliquot of the protein pellet was also counted. Another aliquot of lipid extract was used for separation of galactolipids and glucolipids by thin layer chromatography using 1% borate impregnated slides and chloroform–methanol–water–15 M NH_4H (280:70:6:1) as developing solvent [7]. Phospholipids, glycolipids and neutral lipids were separated by the solvent system of Vitiello and Zanetta [16]. After iodine visualization of each lipid and identification with standards [15], they were scraped and counted with Beckman ready-solv scintillation solution. Proteins were also counted and measured by the Folin methods [8].

Three series of data were obtained: (1) incorporation of [^{14}C]galactose into normal nerve lipids; (2) incorporation of [^{14}C]galactose into saline-injected nerve lipids; (3) incorporation of [^{14}C]galactose into the lipids of the contralateral nerve.

In control nerves, radioactivity incorporation into the homogenate was 4.02% of the added radioactivity in the incubation medium, whereas in saline-injected nerves the radioactivity of homogenate was 5.23% of the added radioactivity. This increased incorporation of label ($0.05 > P > 0.02$) may reflect stimulation of the metabolism of Schwann cells due to the small microinjection injury. For the contralateral nerve, incorporation of label in homogenate was just between the values of control and saline-injected nerve and did not differ significantly from either. For the 3 groups of nerves, incorporation of ^{14}C-label into lipid extract (LE) and proteins (P) was not significantly different, despite a small increase of protein incorporation into injected and contralateral nerves (see Table I). However, the ratio LE/P (dpm/mg P) was significantly decreased ($0.05 > P > 0.02$) in contralateral nerves compared with control nerves.

Table II shows radioactivity incorporation into lipids. [^{14}C]Galactose is incorporated not only into glycolipids but can be metabolized and then incorporated into galactose-devoid lipids such as neutral lipids or phospholipids. Methylation of fatty acids [10] was performed with boron fluoride and counting showed that the radioactivity was found only in the glycerol phase (97.6% of the label) whereas the upper phase containing free fatty acids was not labeled. Hydrolysis of cerebrosides [12] showed that 90% of the radioactivity was associated with sugar, whereas only 4.4% of the label was associated with fatty acids and sphingosine. After chromatography on a borate impregnated slide, 96.4% of the radioactivity associated with cerebrosides occurs as native galactose and only 3.6% is incorporated as glucose.

TABLE I

INCORPORATION OF [^{14}C]GALACTOSE INTO HOMOGENATE, LIPID EXTRACT AND PROTEINS IN CONTROL NERVES, SALINE INJECTED NERVES AND CONTRALATERAL NERVES AFTER 3 h INCUBATION

Results are the mean of 10 values expressed in dpm/mg proteins. Comparisons were made using Student's *t*-test.

	Control nerves	Saline-injected nerves	Contralateral nerves
Homogenate	447,219 ± 69,933	581,645 ± 108,799	523,951 ± 85,454
Proteins (P)	16,810 ± 5297	23,330 ± 3585	21,797 ± 3386
Lipid extract (LE)	48,801 ± 8403	54,262 ± 2367	44,950 ± 3499
LE/P	2.90	2.33	2.06

TABLE II

INCORPORATION OF [^{14}C] INTO LIPIDS OF CONTROL NERVES, SALINE INJECTED NERVES AND CONTRALATERAL NERVES

Results are the mean of 10 values expressed as percentage of incorporation ± S.E.M. Comparisons were made using Student's *t*-test.

Class of lipids	Control nerves	Saline-injected nerves	Contralateral nerves
Sphingomyelin	0.89 ± 0.17	0.66 ± 0.27	0.60 ± 0.11
Phosphatidylcholine	4.72 ± 0.46	5.96 ± 1.55*	5.25 ± 0.65*
Phosphatidylserine	1.19 ± 0.28	3.99 ± 1.72**	3.10 ± 0.40*
Phosphatidylinositol	0.89 ± 0.16	0.67 ± 0.11	0.83 ± 0.10
Phosphatidylethanolamine	1.15 ± 0.27	1.85 ± 0.61	1.61 ± 0.29
Sulfatides	2.08 ± 0.13	4.80 ± 3.12*	7.11 ± 2.93***
Cerebrosides	64.66 ± 5.58	59.43 ± 5.72*	62.03 ± 2.61
Monogalactosyldiacylglycerol	20.45 ± 2.61	18.30 ± 1.02	15.87 ± 0.79**
Neutral lipids	3.97 ± 0.53	3.00 ± 0.47	3.33 ± 0.67

Significance levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control.

Percentage distribution of ^{14}C in control nerves showed that cerebrosides were the most labeled lipids. They incorporated 65% of the total radioactivity of the organic extract and 2/3 of this radioactivity was in hydroxylated cerebrosides. Almost 8% of radioactivity was incorporated into phospholipids and especially phosphatidylcholine.

Comparing the incorporation of radioactivity in saline injected nerves with that in control nerves (Table II), it appears that microinjection is not without effect on Schwann cell lipid metabolism. Indeed, all galactolipids are less labeled than those

of control nerves; on the other hand, phosphatidylserine labeling increases dramatically when compared with control nerves. Incorporation into phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and sulfatide (Sulf) is not affected.

As shown in Table II, incorporation of ^{14}C into lipids of contralateral nerves is also modified as compared with control nerve values. PC, PS and sulfatide incorporation are particularly affected.

These results show that injection of a small quantity (1 μl) of physiological saline into one sciatic nerve can affect the lipid metabolism of the contralateral sciatic nerve in the rat. The technique of endoneural injection has, however, been improved since the studies of Ghabriel and Allt [4] who used glass micropipettes mounted on the needle of a glass syringe. They injected 2 μl of agent with the syringe held firmly by a micromanipulator. At the same time, Dyck et al. [2] assessed the effect of endoneural injection using several procedures. They reported that the technique of subperineural injection itself may produce low rates of segmental demyelination that were significantly above those found in control nerves. Maybe, the inevitable breakdown of perineurium integrity was enough to explain the observed abnormalities. Spencer and colleagues [14] showed that the perineurial window produced pathological changes in underlying fibers. Numerous studies have shown that the contralateral sciatic nerve is affected when the ipsilateral sciatic nerve is drastically severed (section or crush) but this is the first study showing a contralateral alteration after an endoneural injection, which is a very light trauma. Nevertheless, the rats of the control group have been anaesthetized one time whereas the rats with injected sciatic nerves were anaesthetized two times and we cannot exclude the possibility that the effect of number of anaesthetizations can interfere with the effect of the endoneural injection.

After section of the ipsilateral nerve, changes in the contralateral nerves were an increase in weight [17], a decrease in protein content, a decrease in cerebrosides and sulfatides [18], an increase in PC [6], a better ability to regenerate than the control nerve [1] and perturbation of Schwann cells [13]. Because of the experimental timing (quite rapid: 24 h between injection of ipsilateral nerve and incubation of contralateral nerve) it is possible that the effect on the contralateral nerve is caused by a transspinal electrical signal.

We conclude that the contralateral nerve of an injected nerve is not a good control.

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