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The physiological effect of anti-GM1 antibodies on saltatory conduction and transmembrane currents in single motor axons

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Summary

Anti-ganglioside (anti-GM1) antibodies have been implicated in the pathogenesis of Guillain–Barré syndrome, multifocal motor neuropathy and motor neuron diseases. It has been held that they may interfere with saltatory conduction by blocking sodium channels. We tested this hypothesis by analysing action potentials from 140 single nerve fibres in 22 rat ventral roots using external longitudinal current measurement. High-titre anti-GM1 sera from Guillain–Barré syndrome or multifocal motor neuropathy patients, or anti-GM1 rabbit sera were applied to the rat ventral root, where saltatory conduction in single motor fibres was serially observed for 4–12 h (mean 8.2 h). For control experiments, we also tested anti-galactocerebroside (anti-GalC) sera, which causes acute demyelinative conduction block, and tetrodotoxin (TTX), a sodium channel blocker. Conduction block was found in 82% of the fibres treated with anti-GalC sera and 100% treated with TTX, but only in 2% (one out of 44) treated with the patients' sera and 5% (two out of 38) treated with rabbit anti-GM1 sera. All the nodes blocked by anti-GM1 sera revealed intense passive outward membrane current, in the internode just beyond the last active node. This pattern of current flow was similar to that in fibres blocked by demyelination with anti-GalC sera, and quite different from that seen in fibres blocked by reducing sodium currents with TTX. Our findings suggest that anti-GM1 sera neither mediate conduction block nor block sodium channels on their own. We conclude that physiological action of the antibody alone is insufficient to explain clinically observed conduction block in human diseases.

Keywords: anti-GM1 antibody; sodium channel; conduction block; demyelination; external longitudinal current

Abbreviations: anti-GM1 = anti-GM1 ganglioside; anti-GalC = anti-galactocerebroside; CIDP = chronic inflammatory demyelinating neuropathy; TTX = tetrodotoxin

Introduction

Although anti-GM1 ganglioside (anti-GM1) antibodies show elevated titres in the axonal form of Guillain–Barré syndrome, multifocal motor neuropathy and lower motor neuron diseases (Pestronk *et al*., 1988; Pestronk, 1990, 1991; Yuki *et al*., 1990; Yuki, 1994; Visser *et al*., 1995), their exact role in the pathogenesis of these diseases remains elusive (Lange and Trojaborg, 1994; Parry, 1994). Anti-GM1 sera caused conduction block in some studies (Thomas *et al*., 1991; Santoro *et al*., 1992; Arasaki *et al*., 1993; Uncini *et al*., 1993), but not in others (Hughes *et al*., 1985, Harvey *et al*., 1995). Intraneural injection of the sera into the rat sciatic nerve *in vivo* used in many of these studies for bypassing the blood–nerve barrier may traumatize the nerve at the injection site. A control study examining the effect of injecting the vehicle or injection itself is not sufficient to exclude the combined effect of the antibody and trauma, thus confounding the interpretation (Harrison *et al*., 1984).

Takigawa *et al.* (1995), using an *in vitro* model, found that anti-GM1 antibodies increased potassium current elicited by depolarization, and more importantly, blocked sodium channel current irreversibly in the presence of the complement. This led to the hypothesis that sodium channel

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blockade by the antibody may play a pathogenic role in human diseases (Waxman, 1995; Gutmann and Gutmann, 1996). Despite these findings on ion channels, it has not yet been shown whether anti-GM1 antibodies block conduction on their own, or if so, whether the conduction block is due to sodium channel blockade.

External longitudinal current measurements in rat ventral roots provide a unique opportunity to monitor the saltatory conduction in trauma-free single nerve fibres *in vivo*, in order to analyse the mechanism of conduction block (Bostock and Sears, 1978; Kaji and Sumner, 1989; Bostock, 1993). Topical application of the test sera to the root, which is almost devoid of blood–nerve barrier (Lafontaine *et al*., 1982), can reveal the direct physiological effect of an agent (Kaji and Sumner, 1989). Moreover, this allows a selective study of the motor fibre in contrast to the previous *in vitro* studies of the rat sciatic nerve, which contains both motor and sensory fibres (Takigawa *et al*., 1995). We investigated the action of human and rabbit sera with high-titre anti-GM1 antibodies, including the same lot of sera used in the *in vitro* experiment of Takigawa *et al*. (1995).

Material and methods

Antisera

Anti-GM1 rabbit serum

Ten New Zealand white rabbits were immunized three times at 3-week intervals with 1 mg of GM1 and 5 mg of methylated bovine serum albumin as a carrier, in 1 ml of complete Freund's adjuvants containing 5 mg of H37Ra *Mycobacterium tuberculosis*. The two rabbits whose sera demonstrated the highest titres were selected and bled 2 weeks after the third immunization. The titres of the anti-GM1 antibodies were elevated to a serum dilution of 1 : 16 000, and to 1 : 12 000 for IgG and 1 : 2,000 for IgM, using an ELISA (enzyme-linked immunosorbent assay). Despite these high titres, the animals showed no clinical signs of weakness. These sera were the same as those used by Takigawa *et al*. (1995).

Anti-GM1 sera from patients

Anti-GM1 sera were obtained from four patients (Table 1) with inflammatory neuropathies associated with elevated anti-GM1 antibody titres. Patients 1 and 4 had multifocal motor neuropathy with persistent conduction block. Patients 2 and 3 suffered from a severe axonal form of Guillain–Barré syndrome with predominantly motor involvement. The sera from Patients 2 and 3 were obtained before plasma exchange, which significantly improved clinical signs and symptoms.

Anti-galactocerebroside (anti-GalC) rabbit serum

Antiserum against galactocerebroside was prepared by a method similar to that employed to prepare anti-GM1 antiserum. The rabbit had developed severe flaccid weakness.

Antisera and guinea-pig serum were stored frozen at −80°C until used. These sera were thawed immediately prior to application. For supplementation of complement activity, guinea-pig serum (20%, v/v) or fresh human serum from a healthy man (20%, v/v) was added to each antiserum (80%, v/v).

In order to study the physiological action of the complement, we also examined the effect of fresh mixed human and guinea pig sera on the saltatory conduction (Table 1).

Preparation of animals for recordings

We used adult (300–450 g) Wistar rats; anaesthesia was induced by intraperitoneal injection of sodium pentobarbital and maintained by inhalation of Fluothane in oxygen throughout the experiment. Laminectomy was performed between the first lumbar and first sacral vertebrae. Liquid paraffin was filled over the laminectomy site and maintained at 30°C by a thermostatically regulated infrared lamp. One of the most caudal and intact ventral roots was raised onto recording electrodes in the liquid paraffin (Fig. 1). The root was kept intact and extreme care was taken not to stretch it.

Topical application of antisera

A drop of serum (2–3 μl) was applied to the suspended ventral root with a microsyringe pump. The drop was monitored under an operating microscope to ensure that it enveloped the root over a length of 1–2 mm, and that it remained in the same position on the root during the experiment.

Single nerve fibre action potential recording

The fixed electrodes, e1 and e2, were silver wire, 500 μm in diameter and 2 mm apart (Fig. 1). The negative deflection of an all-or-none single nerve fibre action potential recorded at e1 was used to trigger the averaging computer (*see* below). The electrodes, e3 and e4, were of platinum plate, carved to a rectangular cross-section 200 μm wide by 1 mm deep, and placed 500 μm or 400 μm apart. These electrodes were led to the inputs of a differential amplifier (Biotop®, NEC Biomedical, Tokyo, Japan) with a high-pass filter of 50 Hz. Amplified signals were digitized for spike-triggered averaging using a computer (Signal processor 7T18, NEC Biomedical Ltd, Tokyo, Japan).

Nerve fibres in the ventral root were stimulated through a pair of stainless needles inserted in the tail. The 200-μs square-wave voltage pulses were adjusted in intensity, and the position of the electrodes altered, until single biphasic action potentials were registered by amplifier A1. All-or-none behaviour of the potential was confirmed by slightly decreasing the shock intensity.

The electrode pair e3–e4 was slid along the root by a micromanipulator (SM-15, Narishige, Tokyo, Japan) in

Table 1 *Frequency of conduction block*

Serum	Anti-GM1 antibody titre*	Examined roots (*n*)	Number of isolated SNFAPs	Number of blocked SNFAPs
Patient's sera				
Patient 1 + GPS	12 000	5	25	1 (4%)
Patient 2 + GPS	6400	1	4	0
Patient 3 + FHS	800	2	7	0
Patient 4 + FHS	1600	3	8	0
Patients' sera total	11	4	4	1 (2%)
Rabbit sera				
Anti-GM1 + GPS	>12 000	6	38	2 (5%)
Anti-GalC + GPS	12 000†	3	22	18 (82%)
Controls				
FHS + GPS		1	10	0
TTX (1.0 μg/ml)		1	16	16 (100%)

GPS = guinea-pig serum; SNFAP = single nerve fibre action potential; FHS = fresh normal human serum. *Titres shown are of IgM for patient's sera and of IgG for rabbit's sera. †Anti-GalC titre.

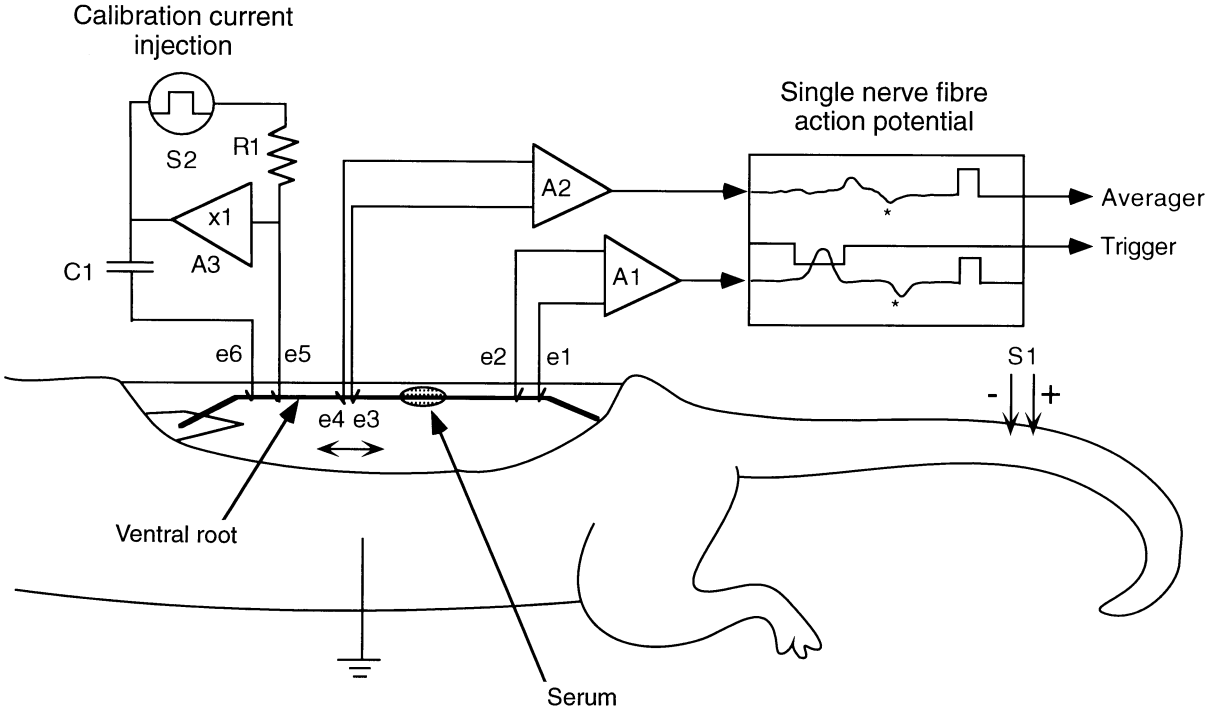

Fig. 1 Recording method. The rat motor nerve in the tail is unique in that a single fibre can easily be stimulated by percutaneous needle electrodes (S1). The antidromically conducting volley was picked up by electrodes, e1 and e2. The single nerve action potential was amplified by A1 and then triggered the sweep of input from electrodes, e3 and e4, which was amplified by A2 and averaged to obtain the external longitudinal current (spike-triggered averaging). The recording electrodes, e3 and e4 were slid along the root for scanning, whereas e5 and e6 were connected to a current source (S2) with a unity-gain amplifier (A3), a resistance (R1) and a capacitor (C1), which generates calibration current (asterisks). A drop of serum was applied to the ventral root, and it was monitored to check that it remained at the same position under the operating microscope. When conduction block was judged to be present by the loss of potential at e3–e4 with intact single nerve fibre action potential at e1–e2, the serum was removed by a pipette and the external longitudinal current was scanned along the segment that had been in contact with the serum. If no block was found, the current was scanned at the end of experiment after the serum removal.

200 μm steps. At each electrode position, a motor nerve action potential conducting antidromically was averaged with the large potential recorded from e1–e2 being used as a trigger (spike-triggered averaging). The potential drop across the gap between electrodes e3 and e4 is proportional to the external longitudinal currents generated by the passing impulse, given the external longitudinal resistance is constant. The latter assumption was confirmed by calibrating these currents with a known longitudinal current pulse, injected via the silver wire electrodes e5 and e6, and analysed further as described previously (Bostock and Sears, 1978).

The transmembrane currents were derived by subtraction of

successively recorded longitudinal currents. These membrane currents were plotted as contours of equal current density in space and time, using linear interpolation by a microcomputer (Macintosh Quadra 650; Apple, USA).

Estimation of membrane properties

If there is a conduction block at a node, the extracellularly recorded waveform of the action potential immediately before the block can be estimated by summing the longitudinal current signals from the node to a proximal part of the root which is unaffected by the impulse (Bostock and Sears, 1978). Because the height of a normal action potential is ~100 mV, the summed longitudinal currents were converted to membrane potential by scaling so that the peak potential at a normal node was 100 mV (Lafontaine *et al*., 1982).

For a patch of nerve membrane, the membrane current i_m is divided into resistive and capacitative components such that

$$i_m = (1/r_m) \times \Delta V_m(t) + c_m \times d/dt \Delta V_m(t) \quad (1)$$

where r_m represents membrane resistance in ohms/centimetre, c_m membrane capacitance in farads/cm, and ΔV_m the displacement of the membrane potential V_m from its resting value. If the segment tested included a node of Ranvier within its length, the membrane currents measured principally comprised those flowing through the node i_n as approximated by

$$i_n = (1/r_n) \times \Delta V_m(t) + c_n \times d/dt \Delta V_m(t) \quad (2)$$

where r_n is nodal resistance in ohms and c_n is nodal capacitance in farads (Lafontaine *et al*., 1982).

In our calculation, membrane potentials and currents were obtained from the experiments, whereas the values of r_n and c_n were estimated from equation (2) using curve fitting, which was adjusted to the experimentally obtained curve of i_m by using least squares. We used Microsoft Excel® Version 5.0 add-in solver function (Microsoft, USA) on a microcomputer (Macintosh Quadra 650; Apple, USA) for computation.

All the procedures have been approved by the Institutional Review Board of Kyoto University School of Medicine.

Results

Normal saltatory conduction

In the normal nerves, external longitudinal currents from a single nerve fibre were abruptly delayed focally at approximately every 1 mm interval (Fig. 2A), where nodes were presumed. By subtracting the adjacent external longitudinal currents, initially outward and then inward transmembrane currents were obtained near the nodes (Fig. 2B). The former represents the depolarizing capacitative current driven by the preceding node and the latter, the sodium action current, triggered by membrane potential reaching the threshold for excitation.

The transmembrane current contour map (Fig. 2C) revealed small outward currents preceded by large inward action currents in time and space. The outward currents were subdivided into focal outward capacitative current at the node (‘a’ in Fig. 2C), which could be too small to be visualized on the contour at some nodes, and a diffuse internodal leakage current (‘b’ in Fig. 2C). These two may become confluent because of the limited spatial resolution (‘a+b’ in Fig. 2C). The internodal conduction time was around 20–30 μs.

We monitored 130 isolated single nerve fibre action potentials for 4–12 h (mean 8.2 h) in 22 ventral roots after applying anti-GM1, anti-GalC, normal human sera supplemented with fresh guinea pig serum or tetrodotoxin (TTX) onto the root (Table 1).

Physiological effect of anti-GM1 sera

Anti-GM1 sera from patients and rabbits were tested in 82 motor fibres. The majority showed no conduction block (Table 1). Figure 3 depicts external longitudinal currents and contour maps in two fibres which were successfully monitored up to 12 h after the application. Neither conduction block nor conduction delay was documented.

Demyelinative conduction block

Anti-GalC serum induced conduction block at the sites of application in 18 out of 22 fibres (Table 1). In these fibres, the internodal conduction time was prolonged adjacent to the unactivated node (Fig. 4A). The remaining four nerve fibres invariably showed increased time for saltatory conduction at the exposed sites. These changes had occurred within 4 h of the application.

In the contour map (Fig. 4A), the internode immediately preceding the blocked node is characterized by a large leakage current (‘b’ in Fig. 4A), which is followed by a small but protracted inward current (‘c’ in Fig. 4A) indicating the presence of a local sodium current that is not sufficient to generate a regenerative sodium action current.

Conduction block induced by TTX

All of the fibres exposed to TTX showed a conduction block within 10 min. Internodal conduction time was prolonged in none of the fibres (Fig. 4B). In contrast to anti-GalC induced block, there was little internodal outward current, but a focus of outward current was found at the blocked node (‘a’ in Fig. 4B). No tail of inward current (indicated by ‘c’ in Fig. 4A) followed the outward current.

Conduction block induced by anti-GM1 sera

Only three out of 82 nerve fibres developed conduction block after exposure to rabbit or patient’s anti-GM1 sera. Internodal conduction time was prolonged in all of these fibres. The contour map at the site of block in one of these fibres (Fig. 4C)

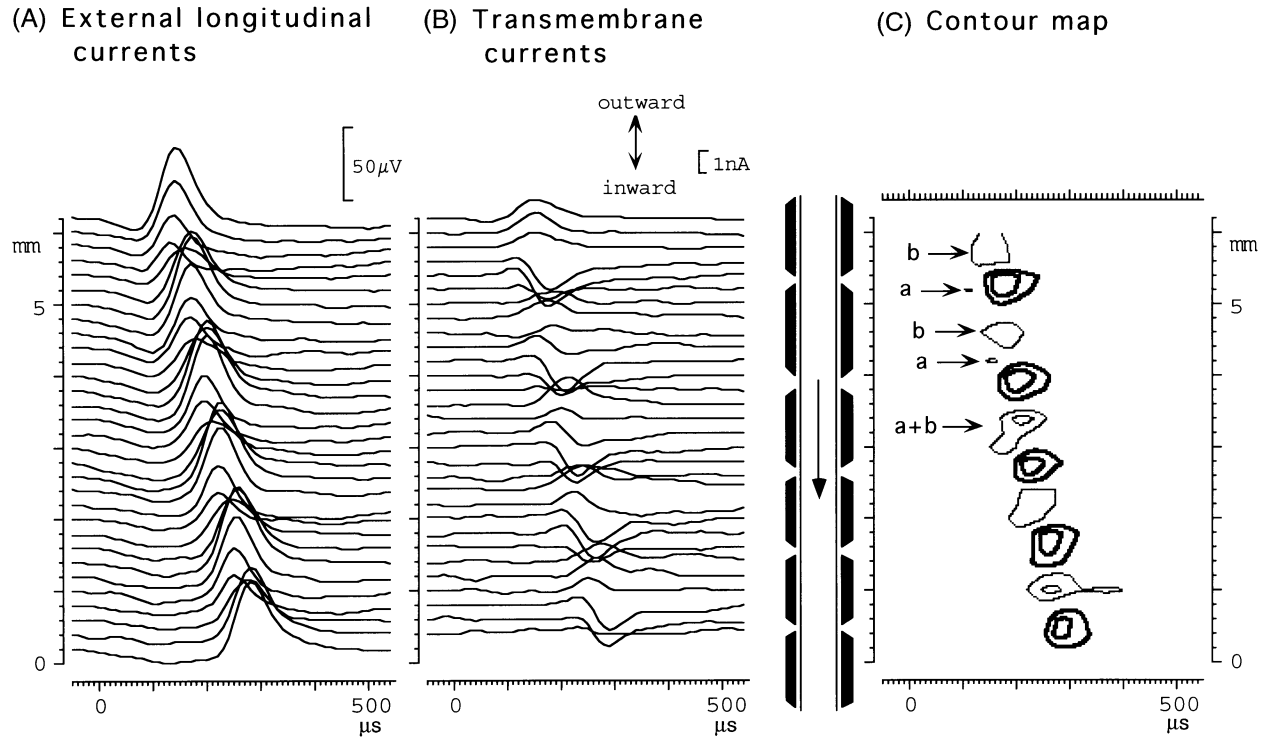

Fig. 2 Saltatory conduction in a normal rat ventral root fibre. (**A**) External longitudinal current. Averaged nerve action potentials (n = 15) recorded at 400-μm intervals along the root. (**B**) Derived transmembrane current calculated by subtracting adjacent records of longitudinal current in **A**. (**C**) Membrane current contour map, plotted on the same distance and time scales. Inward currents are indicated by thick lines, outward currents by thin lines. Contour interval is 0.5 nA. The positions of the nodes of Ranvier are inferred from the derived membrane current record. 'a' indicates the initial outward current at the node, and 'b' denotes internodal outward capacitative current. Sometimes 'a' and 'b' become confluent (a+b), or 'a' was not represented in the contour map because the amplitude was less than a single contour interval (0.5 nA).

resembled anti-GalC induced demyelinative conduction block (Fig. 4A), showing a large outward internodal current (b) followed by a long-lasting tail of inward current (c). This indicates the demyelinative nature of the block with intact internodal sodium channels which generate a local response.

Estimation of the membrane capacitance and resistance

For computation of nodal resistance and capacitance, blocked nodes were defined as the focal sites of increased outward current flow at appropriate positions on the fibre which were estimated on the basis of the internodal distance along the adjacent unaffected segment. For our purpose, the membrane current records must reflect all of the current flowing through the nodes. Thus the membrane currents used were all derived by differentiating external longitudinal currents at two recording sites 400 or 600 μm apart, omitting the intervening records straddling the node.

Normal nodal resistance and capacitance estimated from the nodes blocked by TTX (1.0 μg/ml) (Figs 5A and 6A) were 31 ± 10 MΩ and 3.1 ± 1.0 pF (n = 5). Anti-GalC-exposed nodes showed a decreased mean nodal resistance of 14 MΩ (10–17 MΩ, n = 2) and increased mean nodal capacitance of 34.1 pF (19.6–49.8 pF, n = 3) (Figs 5B and 6B). The site exposed to anti-GM1 patient serum also demonstrated a markedly decreased nodal resistance of 3.9 MΩ and increased nodal capacitance 15.4 pF (Figs 5C and 6C).

Discussion

Using excised single fibres from the sciatic nerve, Takigawa *et al*. (1995) showed in their *in vitro* study that anti-GM1 antibodies at first increased the voltage-dependent potassium current during an action potential, and that in the presence of active complement they decreased the Na^+ current and caused a progressive increase of non-specific leakage current. These findings led to a hypothesis that sodium channel blockage by the antibody is a pathogenic mechanism in multifocal motor neuropathy and Guillain–Barré syndrome or even in amyotrophic lateral sclerosis (Waxman, 1995; Gutmann and Gutmann, 1996).

The present study showed the reverse; the application of the same sera with high anti-GM1 titre blocked conduction in only a small number of fibres, whereas similarly prepared anti-GalC antibodies, mostly composed of IgM macromolecules (Saida *et al*., 1981), did block conduction in the majority of fibres. Our findings do not support the

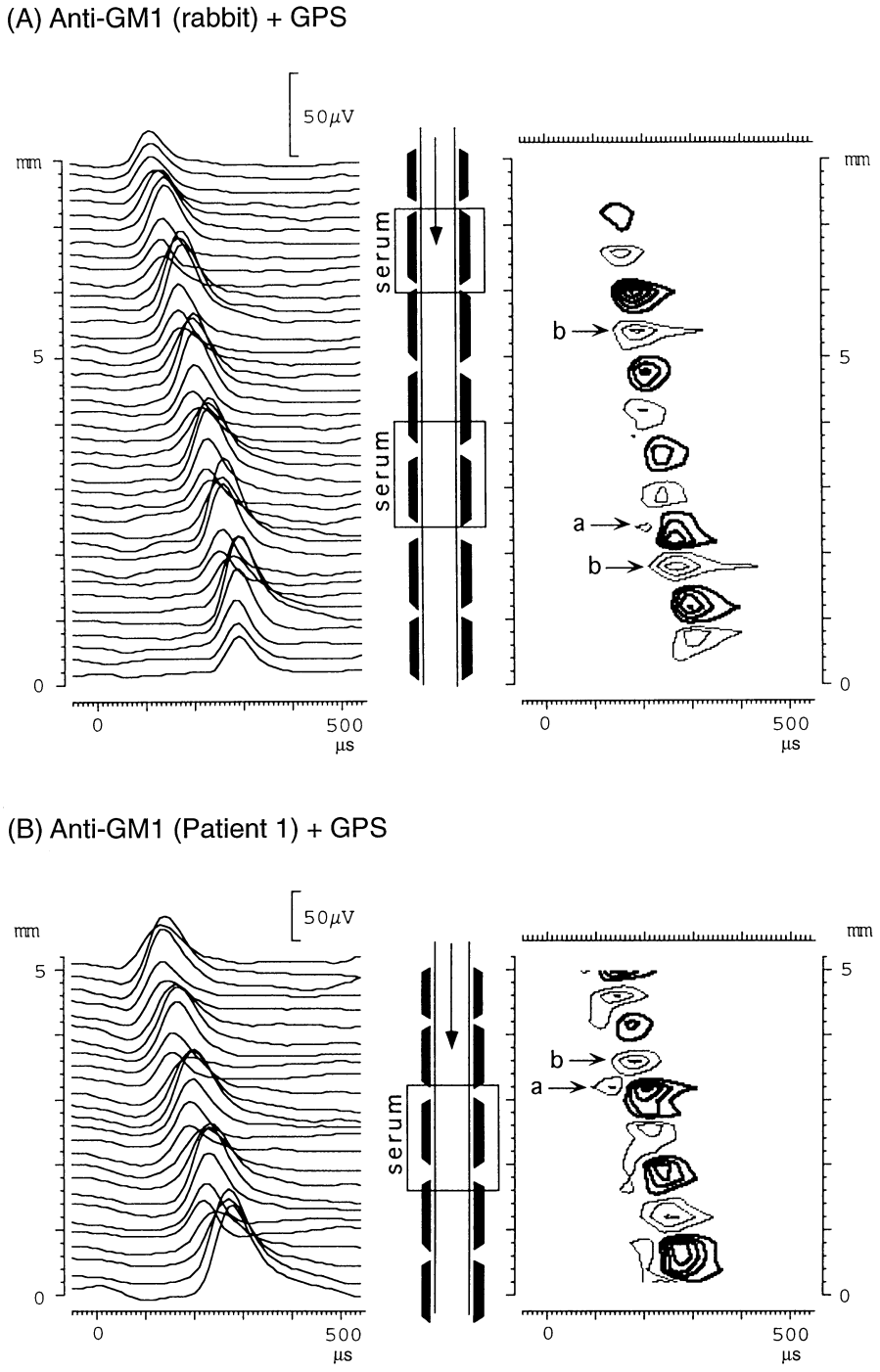

Fig. 3 External longitudinal current recordings and membrane current contour maps from anti-GM1-treated roots. The segments applied with the serum are indicated by rectangles. 'a' indicates the driving current at the node, and 'b' denotes internodal outward capacitative current, as in Fig. 2. (**A**) recording from a root applied with rabbit anti-GM1 serum. (**B**) Recording from a root with patient's serum. GPS = guinea-pig serum.

claim that anti-GM1 antibodies cause conduction block on their own.

Moreover, conduction blocks observed in anti-GM1-treated nerves were associated with a markedly increased internodal outward current. Computation of membrane potentials in relation to transmembrane current showed decreased resistance and increased capacitance of the nodal membrane, which clearly point to the demyelinative nature of the block. Even the subtle internodal inward current, which reflects a local sodium channel response, was preserved. These findings lend little support to the notion that anti-GM1 antibodies block sodium channels.

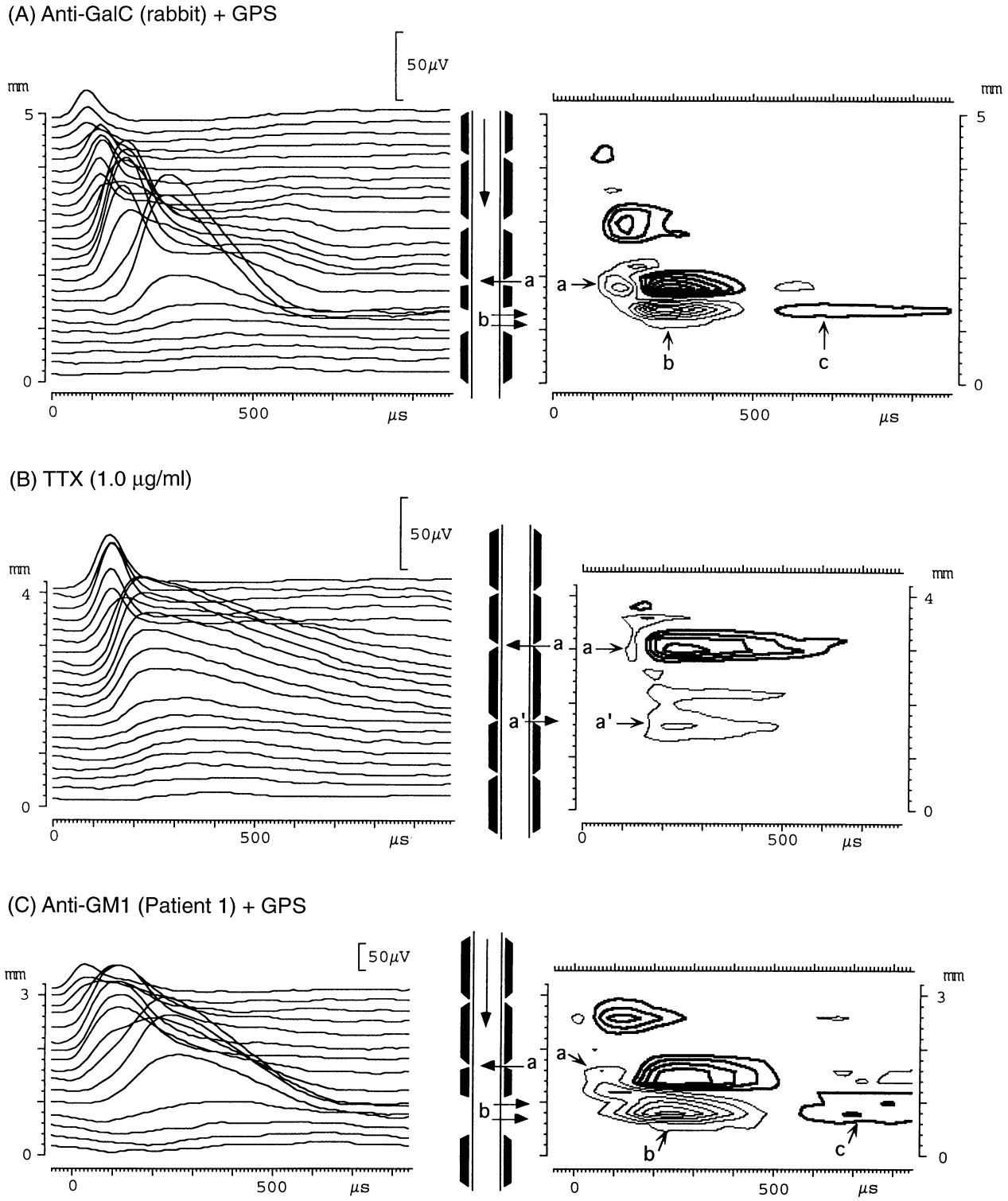

Fig. 4 External longitudinal current recording and membrane current contour map from roots showing conduction block. 'a' indicates the initial outward current at the node, and 'b' denotes internodal outward capacitative current, as in Fig. 2. (**A**) Acute demyelinating conduction block induced 5 h after exposure to anti-GalC serum from rabbit. Contour interval is 0.8 nA. Markedly increased internodal outward current (b) is followed by a long-lasting inward current (c). (**B**) Conduction block induced 10 min after exposure to 1.0 μg/ml TTX. Contour interval is 0.4 nA. (**C**) Conduction block was induced by exposure to anti-GM1 serum from a CIDP patient. Contour interval is 0.3 nA. Markedly increased internodal outward current (b) is followed by a long-lasting inward current (c) as in **A**. GPS = guinea-pig serum.

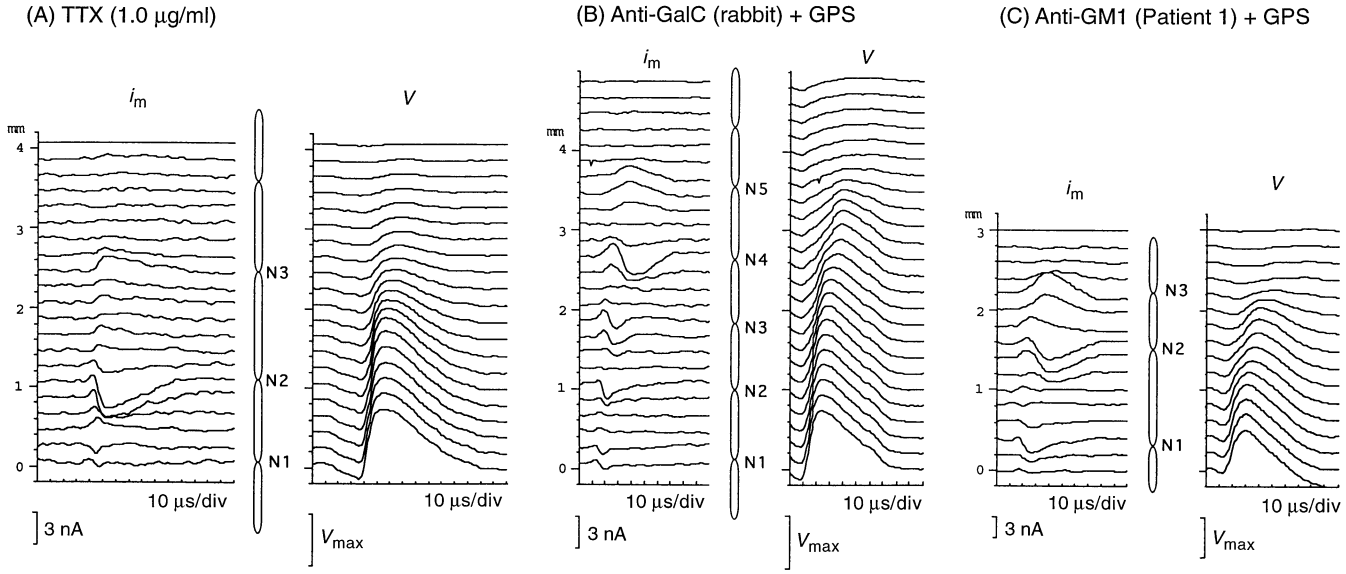

Fig. 5 Membrane current (i_m) and extracellular potential (V) at successive sites along a ventral root fibre focally exposed to 1.0 μg/ml TTX (**A**), anti-GalC serum from rabbit (B), anti-GM1 serum from a CIDP patient (**C**). The direction of propagation is upward and the positions of the nodes of Ranvier are inferred from the derived membrane current record. In these traces, upward and downward deflections show outward and inward membrane current, respectively. The potential calibration for these traces is the full action potential V_{max} (= 100 μV) recorded in the portion of the root in which conduction is normal. GPS = guinea-pig serum.

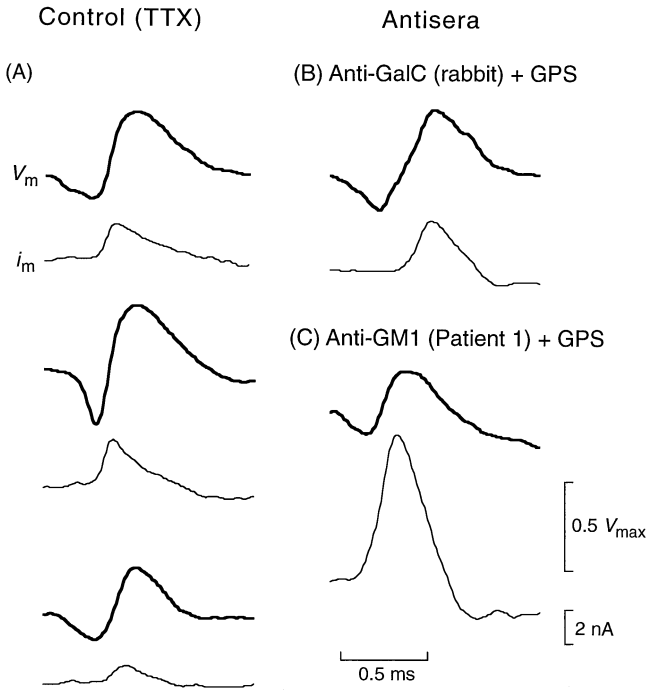

Fig. 6 Membrane current and extracellular potential at nodes where conduction failure is due to TTX (**A**), anti-GalC serum (**B**) or anti-GM1 serum (**C**). Current, voltage and time scales are the same for all pairs of recordings.

The other physiological studies on anti-GM1 antibodies also showed divergent findings. Thomas *et al*. (1991) immunized rabbits with GM1 or with Gal(β 1–3)GalNAc-BSA. Development of antibodies to these antigens was associated with a fall in the ratio of the amplitudes of the compound muscle action potential evoked by proximal versus distal stimulation of the sciatic nerve. Pathological studies revealed mild axonal degeneration and immunoglobulin deposits at the nodes of Ranvier in peripheral nerve. By contrast, the rabbits used for preparing anti-GM1 antibodies in this and the previous (Takigawa *et al*., 1995) studies showed no clinical signs of neuropathies.

The following two studies using intraneural injection into the rat sciatic nerve reported that anti-GM1 sera produced conduction block with a significant fall in the amplitude ratio (Santoro *et al*., 1992; Uncini *et al*., 1993). The degree of the block in these studies, however, was modest as compared with that in studies using anti-GalC sera (Sumner *et al*., 1982). Interestingly, the later study (Uncini *et al*., 1993) included anti-GM1 sera of a comparable titre from a patient with spinal progressive muscular atrophy, which did not cause conduction block. These authors ascribed this discrepancy to the different affinity or specificity of the antibodies. Another supportive piece of evidence was reported by Arasaki *et al*. (1993), who found a minor reduction of compound nerve action potential amplitudes in excised and desheathed rat sciatic nerves. Harvey *et al*. (1995), on the other hand, failed to induce conduction block with an intraneural injection technique, despite the binding of anti-GM1 antibody to the node of Ranvier. Similar negative results was also reported by others (e.g. Hughes *et al*., 1985).

These experimental models are significantly different from the human disease. For instance, a defective blood–nerve barrier would permit a continuous passage of serum constituents into the human nerve, so that nerve fibres would undergo protracted challenge of the antibody. By contrast, the model used in the present study only allows the monitor-

ing of physiological changes up to 12 h after topical application of the sera. It might therefore be argued that chronic exposure to anti-GM1 antibodies results in more extensive demyelination or more frequent conduction blocks.

This, however, is not likely on the following grounds. First, Harvey *et al*. (1995) examined the action of anti-GM1 IgG and IgM antibodies using an intraneural injection for a longer period; despite the immunohistochemical evidence of the antibody binding to the nodes of Ranvier, no conduction block was seen during the follow-up period of up to 8 days. Serum from a rat with experimental allergic neuritis could block conduction within 1 day after injection. Secondly, Takigawa *et al*. (1995) observed the effect on ion channels within just a few hours using the same sera as ours. The putative action of anti-GM1 antibodies would be expected to take place within a comparable observation period in our model. By measuring membrane currents, which are affected prior to the development of conduction block, in the present study we should have been able to detect a subtle physiological effect, if any, at least within several hours, as was the case for anti-GalC antibodies.

The finding that anti-GM1 antibodies increased potassium current, as reported previously (Takigawa *et al*., 1995), is most likely due to paranodal demyelination, which exposes voltage-dependent potassium channels. After extensive searches for evidence of sodium channel blockage, we were unable to confirm the findings of acute sodium current reduction. In the previous study, Takigawa *et al*. (1995) used an incubating bath with no metabolic supplements, which may have inhibited the sodium–potassium pump. This not only tends to decrease the sodium concentration gradient across the membrane, but also predisposes the axons to damage by reverse operation of the sodium–calcium exchanger (Stys *et al*., 1992). The axonal membrane may be more prone to be damaged than in our model because of this metabolic compromise. As was stated in the study of Takigawa *et al*. (1995), the antibody and the complement may disrupt the axonal membrane, where GM1 is abundant (Schwerer *et al*., 1986). If so, the irreversibly decreased sodium current could be secondary to a combined effect of sodium–potassium pump inhibition and axonal damage, which subsequently leads to a decrease in the sodium ion concentration gradient across the membrane, causing the sodium current loss. Thus the sodium channels need not be targeted specifically, although the antibody may prime the axonal membrane to be damaged by the complement.

While the present study demonstrated the lack of significant physiological action of the anti-GM1 antibody, there has been abundant evidence that the antibody occurs more frequently in certain disease conditions (Pestronk *et al*., 1988; Pestronk, 1990, 1991; Yuki *et al*., 1990; Yuki, 1994;Visser *et al*., 1995). If anti-GM1 antibodies ever play a role in the human disease, what are the remaining possibilities?

Multifocal motor neuropathy

Multifocal motor neuropathy is a treatable condition with chronic insidious onset characterized by multifocal weakness and persistent conduction block (Parry, 1985; Parry and Clarke, 1988; Pestronk *et al*., 1988). Despite the predominant motor involvement, it occasionally presents minor sensory symptoms, and may be regarded as a focal manifestation of chronic inflammatory demyelinating neuropathy (CIDP) (Thomas *et al*., 1996). Affected muscles usually show fasciculation and myokymia, phenomena of increased excitability in the motor nerve (Roth *et al*., 1986; Parry and Clarke, 1988). Although elevated titres of anti-GM1 antibodies were originally reported (Pestronk *et al*., 1988), their incidence has been variable in recent reports (Krarup *et al*., 1990; Pestronk, 1991; Kaji *et al*., 1992, 1993; Kornberg and Pestronk, 1994; Lange and Trojaborg, 1994; Parry, 1994; Bouche *et al*., 1995). Because so many patients lack the antibody, doubt has been cast upon its pathogenic significance (Lange and Trojaborg, 1994; Parry, 1994; Thomas *et al*., 1996).

An unusual feature in multifocal motor neuropathy is the persistence of conduction block. Experimental conduction block induced by anti-sera is rapidly reversed by remyelination (Saida *et al*., 1980). Similarly, the clinical course is remittent in the majority of patients with CIDP, relapses being followed by remissions. In contrast, most of the untreated patients with multifocal motor neuropathy present a monophasic course with little signs of remission. Pathological findings at the site of lesion include demyelination with little evidence of remyelination (Kaji *et al*., 1993), which is consistent with persistent conduction block.

Immunoglobulin deposits were found at nodes of Ranvier in biopsied nerve from a patient with multifocal motor neuropathy (Santoro *et al*., 1992), in nerves from rabbits immunized with GM1 (Thomas *et al*., 1991), and in rat sciatic nerves treated with anti-GM1 antibodies (Harvey *et al*., 1995). If such deposits occur at already demyelinated axons, membrane-bound immunoglobulins, especially macromolecular IgM, may interfere with remyelination, because Schwann cell process may not be able to recognize axonal surface antigens for remyelination (Wood *et al*., 1990). This inhibition of remyelination may be selective for motor axons, if the antibody has higher affinity for motor than sensory axons (Thomas *et al*., 1990; Corbo *et al*., 1992; Ogawa-Goto *et al*., 1992; Yoshino *et al*., 1992).

For these antibodies to gain access to the demyelinated segment, there must be persistent impairment of the blood–nerve barrier. Indeed the blood–nerve barrier was disrupted at the lesion site, with persistent conduction block, in a patient with multifocal motor neuropathy by MRI studies (Kaji *et al*., 1994). Alternatively, this constant exposure of the axonal GM1 antigen to the immune system might have elicited the antibody.

Axonal Guillain–Barré syndrome

High-titre anti-GM1 antibodies were frequently reported in axonal or motor-dominant Guillain–Barré syndrome (Yuki

et al., 1990; Yuki, 1994; Illa *et al*., 1995; Ogino *et al*., 1995; Visser *et al*., 1995). These antibodies are mostly of IgG rather than IgM class. This clinical type of Guillain–Barré syndrome is often associated with electrophysiological signs of axonal degeneration in motor fibres and muscle denervation. Anti-GM1 antibodies may play a pathogenic role here, if they predispose the axonal membrane to disruption, as discussed previously.

However, the present study did not show convincingly that axonal damage was taking place. When focal membrane damage disrupts the continuity of the axon, the distal and proximal nerve stumps remain viable for a few days. The axonal damage is therefore detected as focal conduction block in our model, albeit being irreversible. The lack of such findings may indicate that the antibody alone is not potent enough to damage axons *in vivo*. Other soluble inflammatory mediators must operate in concert. Tumour necrosis factor-α is one of these mediators and intraneural injection of it produces inflammatory vascular changes within the endoneurium, together with axonal degeneration and mild demyelination (Redford *et al*., 1995). Of course, cellular immune responses may also contribute to axonal damage. Selective vulnerability of the motor fibres here may also be explained by the antigenic difference (Thomas *et al*., 1990; Corbo *et al*., 1992; Ogawa-Goto *et al*., 1992; Yoshino *et al*., 1992) or the biophysical difference (Burke *et al*., 1997).

As such, it is conceivable that anti-GM1 antibodies play a pathogenic role in axonal damage in these disease conditions. In the experimental models discussed above, however, nerve fibres are exposed to a bolus of antibodies at a high concentration, but for a brief period. This passive transfer of the antibody alone may not be the optimum system in order to study such subtle, cumulative electrophysiological effects as axonal damage.

In conclusion, from evidence accumulated thus far, it is premature to conclude that anti-GM1 antibodies can exert any physiological action on their own in human disease.

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