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TEMPERATURE ACCLIMATION EFFECTS ON CARP NERVE: A COMPARISON OF NERVE CONDUCTION, MEMBRANE FLUIDITY AND LIPID COMPOSITION

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Summary

1. This paper describes the effects *in vitro* of temperature (5–35°C) on the conduction properties of vagus nerves from freshwater carp *Cyprinus carpio* L., either cold- (8°C) or warm- (28°C) acclimated. The results are related to changes in the physical state and lipid composition of brain membrane fractions.

2. The temperature dependence of the conduction velocity of the *C* (unmyelinated) component of the compound action potential (AP) were determined using Arrhenius plots. The relationship between log time-to-peak AP and the reciprocal of absolute temperature (1/K) is best described by two linear components. The grouped data for the warm-acclimated group had a break point at 23.6°C. At temperatures above 24°C the activation energy E_a was 18.3±8.33 kJ mol^{-1} and below 24°C E_a was 49.7±3.78 kJ mol^{-1}. The break-point for cold-acclimated nerves was 17.4°C with E_a values of 41.2±2.65 and 13.2±3.63 kJ mol^{-1} below and above this temperature, respectively.

3. The Arrhenius plots of the fast-conducting *A* (myelinated) component of the AP for the warm- and for the cold-acclimated group were better fitted by two linear relationships with E_a values of 42.0±2.16 and 86.9±4.55 kJ mol^{-1} above and below the break at 13.1°C for the warm- and cold-activated nerves, respectively, and E_a values of 18.0±5.45 and 58.8±4.08 kJ mol^{-1} above and below the break point, 19.3°C, for warm- and cold-acclimated nerves, respectively.

4. Steady-state fluorescent polarization of 1,6-diphenyl-1,3,5-hexatriene-(DPH) labelled synaptosomal and myelin fractions of carp brain indicated partial homeoviscous adaptation in the membranes. Since there were no appreciable differences in their fatty acids, changes in membrane composition other than in the phospholipid fatty acids presumably occurred.

Introduction

Many animals have evolved a versatile physiology which allows them to adapt to

Key words: thermal acclimation, nerve conduction, membrane lipids, fluidity, *Cyprinus carpio*.

environmental changes, for example to the temperature changes experienced between summer and winter. Compensatory mechanisms help offset the direct effects of temperature and thus maintain optimal operation. There are many reports of acclimitization in eurythermal teleost fish at several levels of organisation, from the molecular to the integrated behaviour of the whole organism. Generally the process being studied is faster or more active at any given temperature in cold-acclimated than in warm-acclimated animals. For example, skeletal muscle can show alterations in myofibrillar ATPase activity resulting in cold-acclimated muscle contracting more rapidly, and thus more effectively, at lower temperatures (Johnston *et al.* 1975; Johnston and Altringham, 1985; Heap *et al.* 1985).

At the level of membrane structure and function, acclimatory changes have been described in the fatty acid composition of goldfish (*Carassius auratus*) brain synaptosomes (Cossins, 1977) and garfish (*Lepisosteas osseus*) peripheral nerve (Friedman *et al.* 1986), in the fluidity of goldfish synaptosomes (Cossins, 1977) and in amino acid transport in goldfish intestine (Mepham and Smith, 1966). The extent to which the function of excitable membranes adapts is, however, less clear (Macdonald, 1989, 1990). The data available are, with few exceptions, limited to comparative studies of closely related species from distinct thermal environments, e.g. the electrophysiology of nerve and muscle membranes of frogs (Schwartz, 1979) and neuromuscular function in teleost fish (J. A. Macdonald *et al.* 1988).

Electrophysiological studies of excitable cells from a single species subjected to temperature adaptation include central neurones in *Helix aspersa*, skeletal muscle fibres of the green sunfish *Lepomis cyanellus* and the extraocular muscle of carp *Cyprinus carpio* (Langley, 1979; Klein and Prosser, 1985; Klein, 1985; Harper *et al.* 1989). However, there appear to be no accounts of thermal adaptation of neuronal function in a vertebrate species, which is the main concern of this paper. Here we report the effects of temperature acclimation on the temperature coefficient of nerve conduction in an *in vitro* vagus nerve preparation from warm- (28°C) and cold- (8°C) acclimated carp, and we also describe the changes in the lipid composition and membrane fluidity of brain tissue.

A preliminary report of the electrophysiological results has been published (Hancock et *al.* 1987).

Materials and methods

Thermal acclimation

Freshwater carp *Cyprinus carpio* L. (length approx. 25 cm; mass approx. 250 g) were obtained from Newhay fisheries, Selby, Yorkshire. They were maintained at 14°C in tanks of running fresh tap water for 2–3 weeks. The temperature was then changed over 5–7 days to the target acclimation temperatures; warm (28°C) or cold (8°C). These acclimation temperatures were chosen since they were the preferred temperatures for cold- and warm-acclimated fish when exposed to a thermal gradient (Penney and Goldspink, 1981). The fish were then maintained

for at least 3 weeks at their acclimation temperature before being used in experiments, as this has been shown to be the (minimum) period for acclimation of myofibrillar ATPase (Heap *et al.* 1985).

Throughout the maintenance and acclimation periods all fish were fed daily until satiety with a commercial fish pellet and kept under a 12 h:12 h light:dark photoperiod. Adequate feeding is important as starvation has been shown to inhibit the acclimatory response of myofibrillar ATPase (Heap *et al.* 1985).

Myofibrillar ATPase activity

It was important to confirm that the myofibrillar ATPase activity in the experimental animals showed alterations similar to those reported in previous studies (Johnston *et al.* 1975; Heap *et al.* 1985). A portion of the white epaxial muscle was removed from several animals and assayed for its specific activity over the temperature range 5–30 °C. The methods used to assay for the enzymic cleavage of ATP were as described by Heap *et al.* (1985).

Tissue preparation

Fish were stunned by a blow to the head and killed by transection of the spinal cord and pithed rostrally and caudally. The nerve dissection was then carried out on an ice-cold platform. The vagus nerve trunk branches supplying the gill arches and viscera were quickly removed. The isolated trunk was cleared of surrounding connective tissue. White epaxial muscle was also removed at this stage for ATPase assay.

Histological processing

After removal from the animal, small sections of the nerve trunks were fixed with 2.5 % gluteraldehyde/2 % paraformaldehyde in 0.1 mol l^{-1} cacodylate buffer solution, pH 7.3 (for approximately 4 h), then transferred to 0.1 mol l^{-1} cacodylate buffer (overnight). They were then placed in Millonig's (1962) phosphate-buffered osmium tetroxide 1 % (1 h), dehydrated and embedded in Araldite resin. Thin sections were cut so the nerve fibre profiles were in transverse section. The sections were stained in 2 % aqueous uranyl acetate (15 min) and viewed and photographed using an electron microscope.

Electrophysiological measurements

The central length of the isolated nerve trunk was placed on a metal platform covered in Parafilm and lens tissue. The temperature of the thermode was kept constant ±0.2 °C at values between 5 and 35 °C by a circulating ethylene glycol/water mixture through a tortuous channel cut into the block. The ends of the nerve were hooked on stimulating and recording electrodes (bipolar platinum, inter-electrode distance approximately 2 mm). The nerve length distal to the central recording electrode was crushed to obtain monophasic recording conditions and a grounded monopolar electrode was positioned close to the stimulating electrodes to minimise stimulation spread. The stimulating and

recording sites were sealed in a petroleum jelly/paraffin oil mixture to enclose mechanically, stabilise and prevent desiccation of the nerve trunk. The central, major portion of the nerve was superfused with a teleost Ringer's solution (adapted from Shuttleworth, 1972) containing (in mmol l^{-1}): NaCl, 140; KCl, 2.7; $CaCl_2$, 1.5; $NaHCO_3$, 15; and Hepes, 25. The pH was adjusted to 7.5 by addition of 1 mol l^{-1} NaOH and the solution was saturated with oxygen immediately before use.

Compound action potentials (APs) were evoked using square-wave, constant-voltage pulses of 20–100 μs and 100 μs to 1 ms duration for the *A* and *C* (unmyelinated) components, respectively. The evoked electrical activity was recorded using an a.c.-coupled headstage and amplified (Neurolog UK). The signal was filtered (final bandwidth 0.1 Hz to 50 kHz) and averaged (8×0.5 Hz on a Neurolog averager). The output from the averager was read out onto a pen recorder to give a permanent record which was used for subsequent analysis.

Membrane fluidity measurements

Three fish from each acclimation group were killed, and the brains rapidly dissected out and cleared of fatty tissue. The procedures for the preparation of synaptosome and myelin fractions were those described by Cossins (1977) and Cossins and Macdonald (1984). Fluorescence polarization measurements were made using a Perkin Elmer LS-3 fluorimeter using 1,6-diphenyl-1,3,5-hexatriene (DPH) as the fluidity probe, as described by A. G. Macdonald *et al.* (1988).

Statistical analysis and calculation of activation energy

Data are presented as a mean±one standard error: number of samples, *N*, in brackets.

We used the mathematical procedure described by Jones and Molitoris (1984) to fit and characterise the Arrhenius plots of myofibrillar ATPase and nerve conduction. This method was used to indicate where the data were better described by two linear regressions rather than by one, and also to identify the point of change of slope.

The activation energy (E_a) was used to characterise the temperature dependence of the conduction process:

$$E_a = \left(\frac{\boldsymbol{R}T_1T_2}{T_2 - T_1}\right) \ln \frac{X_2}{X_1},$$

where X_1 and X_2 are the values of the experimental parameter at the lower and higher absolute temperatures, respectively, and $\boldsymbol{R}$ is the gas constant (8.314 J K^{-1} mol^{-1}).

All other data were compared using Student's *t*-test.

Results

The myofibrillar ATPase activity was higher in white epaxial muscle from cold-

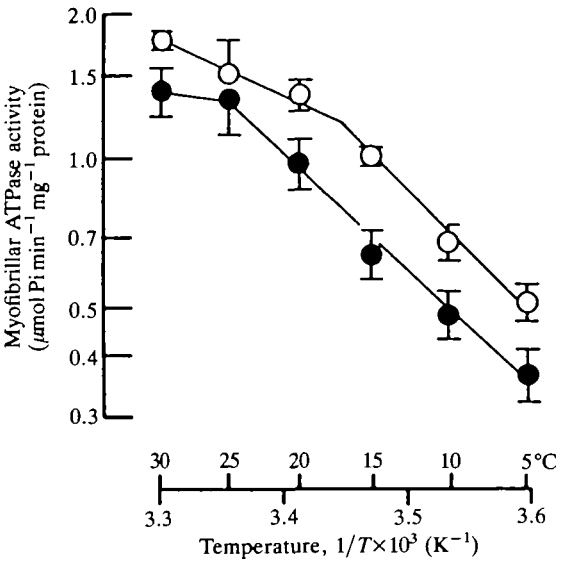

Fig. 1. Myofibrillar ATPase activity of white epaxial muscle from cold- (○) and warm-acclimated (●) fish (N=6 and 5, respectively). The ATPase activity was assayed at reaction temperatures in the range 5–30°C. At an incubation temperature of 20°C the myofibrillar ATPase activities were 1.367±0.109 and 1.085±0.102 μmol phosphate min^{-1} mg^{-1} protein for muscle from cold- and warm-acclimated fish, respectively.

as compared to warm-acclimated fish throughout the range of assay temperatures 5–30°C (Fig. 1), and the enzyme activities are in good agreement with previous reports.

The isolated nerve trunks remained in good condition for up to 6 h when superfused with Ringer's solution, as judged by the amplitude and conformation of the compound action potential (AP). Temperature was changed in steps of approximately 4°C and the preparation was allowed to equilibrate for at least 5 min before recording. We examined the steady-state changes in nerve conduction. Rapid temperature changes may cause transitory changes in electrical properties, as reported for guinea pig cardiac cells (Cavalie *et al.* 1985). No apparent difference was seen in the results when temperature was increased and decreased in a preparation. At least eight test temperatures were recorded in each preparation.

The two branches of the vagus supplying the gill arch and viscera had distinct nerve fibre type distributions and electrophysiological properties. The branch supplying the gill arch was characterized by a large fast-conducting component corresponding to A (myelinated) fibres. This waveform was recruited at low stimulus intensities, typically 10 V, 100 μs, for full activation. Inspection of electron micrographs (Fig. 2) shows that this nerve trunk contains many large-diameter myelinated fibres which were probably involved in such activity. Despite repeated attempts (N>10) no C (unmyelinated) fibre component could be

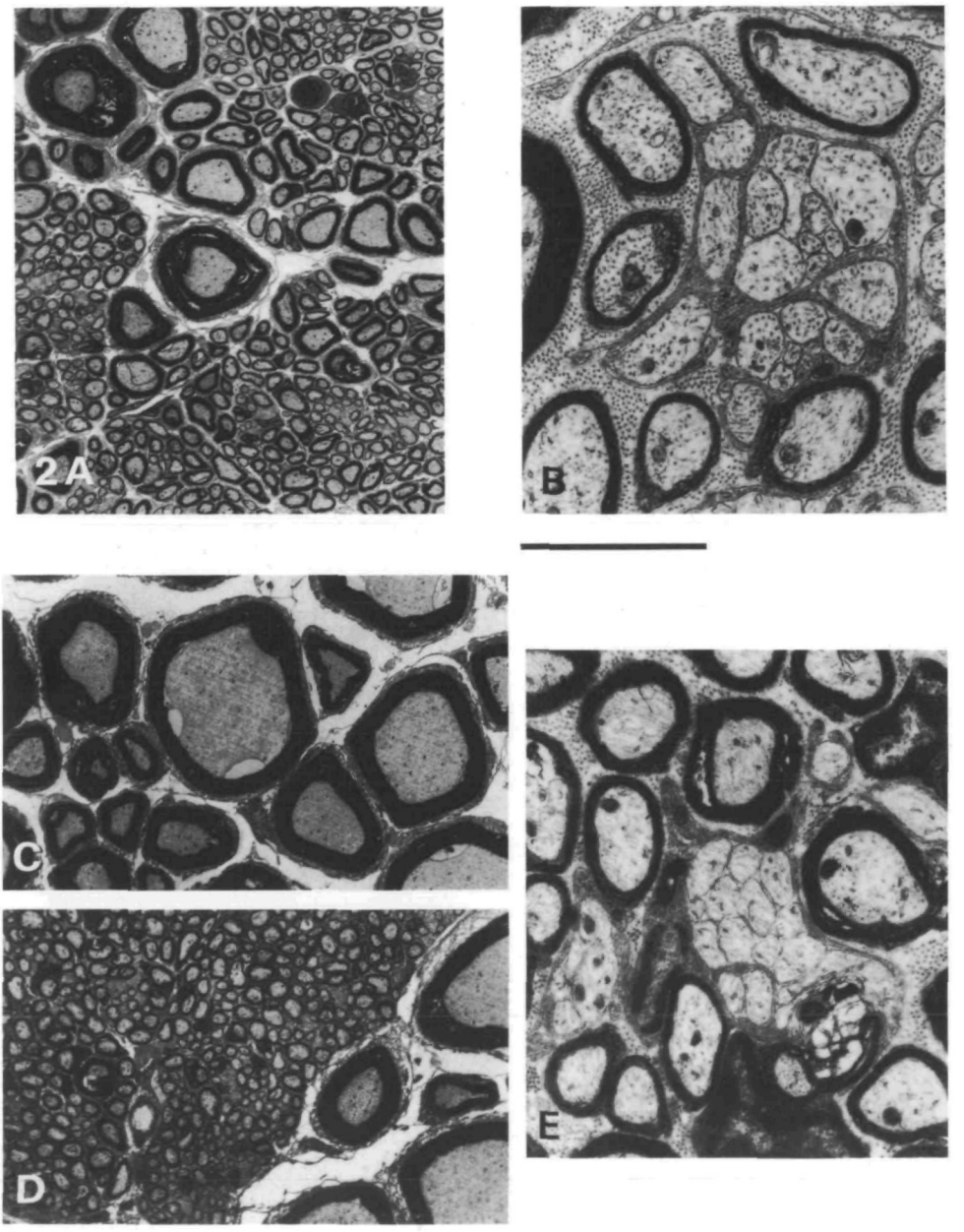

Fig. 2. Electron micrographs of the visceral (A,B) and gill arch (C–E) branches of the vagus nerve trunk from a warm-acclimated carp. (A) Low-power micrograph illustrating a large proportion of small myelinated fibres in this nerve compared with the gill arch vagus (C,D) at the same magnification. C and D show two aspects of the gill arch vagus. A region that contains large myelinated fibres (C), (up to 20 μm in diameter) is anatomically distinct from that containing predominantly small-diameter myelinated fibres (D). (B,E) High-power electron micrographs of unmyelinated fibre bundles. The visceral vagus (B) contained many more of these in a given field than the gill arch nerve (E). Scale bar, A,C,D 20 μm; B,E 2 μm.

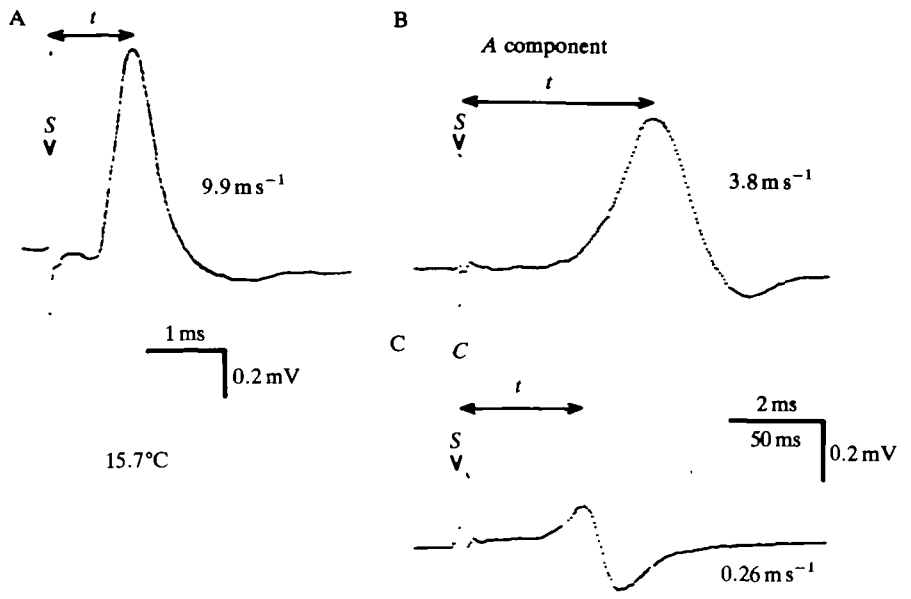

Fig. 3. *A* and *C* components of the compound action potential recorded from the vagus. (A) The fast-conducting (*A* fibre) myelinated component of the gill arch vagus (cold-acclimated); (B,C) Examples of *A* and *C* fibre action potential components recorded from the visceral vagus (warm-acclimated). Stimuli 10 V, 100 μs; 24 V, 1.0 ms (twice full threshold) for *A* and *C* components, respectively, applied at 15.7°C for both groups. Conduction velocity was calculated from the quotient of conduction distance and latency (*t*). The records are the average of eight sweeps at 0.5 Hz. *S*, stimulus artefact.

recorded from this nerve. In contrast, both *A* and *C* fibre volleys could be recorded from the visceral vagus (Fig. 3) (the *A* wave at voltages and pulse durations as low as for the gill arch branch of the vagus, but the *C* wave requiring more than 50 V at 100 μs and 20 V at 1 ms for full recruitment). Electron micrographs (Fig. 2A,B) of the visceral vagus revealed that, by comparison with the gill arch branch, it contained a higher proportion of unmyelinated fibres. Additionally, although there were many myelinated fibres, they were generally of smaller diameter. Thus, cursory morphological analysis of the nerve composition of the vagal branches used is in line with the electrophysiological recordings.

Conduction velocity

The most marked effect of temperature was on the conduction velocity of the APs. Increasing temperature decreased the time to the inflection and peak of the response, indicating greater maximum and mean conduction velocities of both *A* and *C* components. In many instances, particularly for the gill arch branch of the vagus, the length of isolated nerve was small so that accurate conduction velocity measurements were not always possible. Instead, the time-to-peak of the action potential (the reciprocal of which gives an index of conduction velocity) was

measured. In those nerve trunks in which conduction distance was measured satisfactorily we found that the conduction velocities of the nerve trunks from cold-acclimated fish were faster at low temperatures than those of their warm-acclimated counterparts. Data are presented as the mean conduction velocity ($m\,s^{-1}$) 28°C/8°C: *C* fibre AP (visceral vagus) 28°C-acclimated, 0.50/0.12; *C* fibre 8°C-acclimated, 0.38/0.16 (N=4,4); *A* fibre AP (gill arch vagus) 28°C-acclimated, 21/3.7; *A* fibre 8°C-acclimated 25/7.5 (N=2,3). Individual examples of the effect of temperature on the time-to-peak of *A* and *C* fibre components of the action potential for nerves from warm- and cold-acclimated fish are shown as Arrhenius plots (Fig. 4). The effects of temperature on the conduction of the *A* and *C* fibre APs for nerves from warm- and cold-acclimated fish are shown in Figs 5 and 6.

The data were better described by two linear regressions than by one. Considering the acute effect of cooling the nerves from the warm-acclimated fish, conduction velocities of the *A* and *C* fibres are reduced to 0.22 and 0.29 of their respective values at 28°C by a 20°C decrease to 8°C. In the animals acclimated to 8°C, conduction velocities at 8°C are 0.31 and 0.47 of those seen at 28°C for *A* and *C* fibres, respectively. The *C* fibre AP 'break' temperature was higher for nerves from warm-acclimated fish than for those from cold-acclimated fish. For *A* fibres, however, the break temperature was lower for warm- than for cold-acclimated nerves.

The apparent activation energies (E_a) of the conduction process were calculated from the fitted slopes of the Arrhenius plots. E_a values for *C* fibre conduction at temperatures above the break were similar for both groups: 18.3±8.3 kJ mol^{-1} for

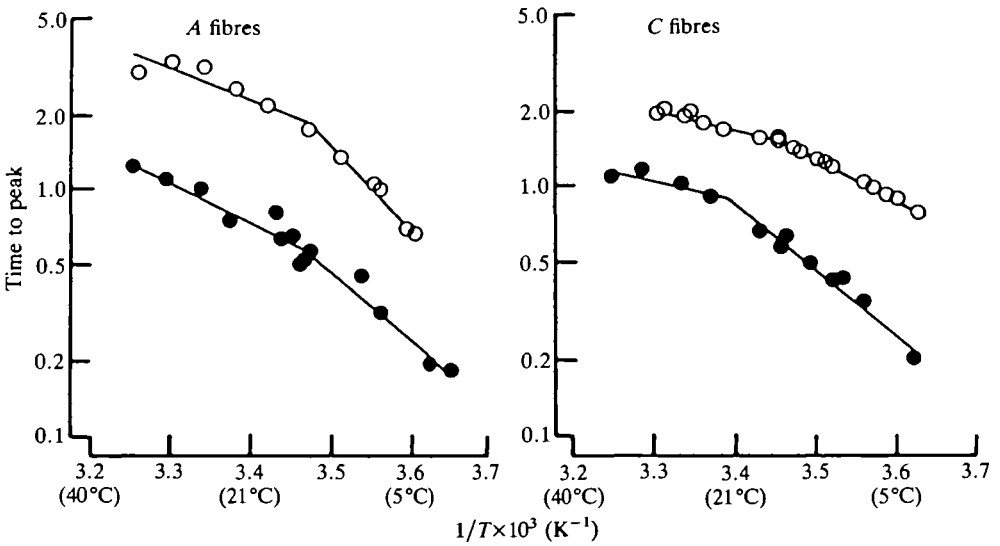

Fig. 4. Examples of Arrhenius plots of the reciprocal time- to peak-compound action potential for individual nerve trunks. Values are expressed as a fraction of that measured at the acclimation temperature against 1/temperature (K). Cold-acclimated fibres (○); warm-acclimated fibres (●).

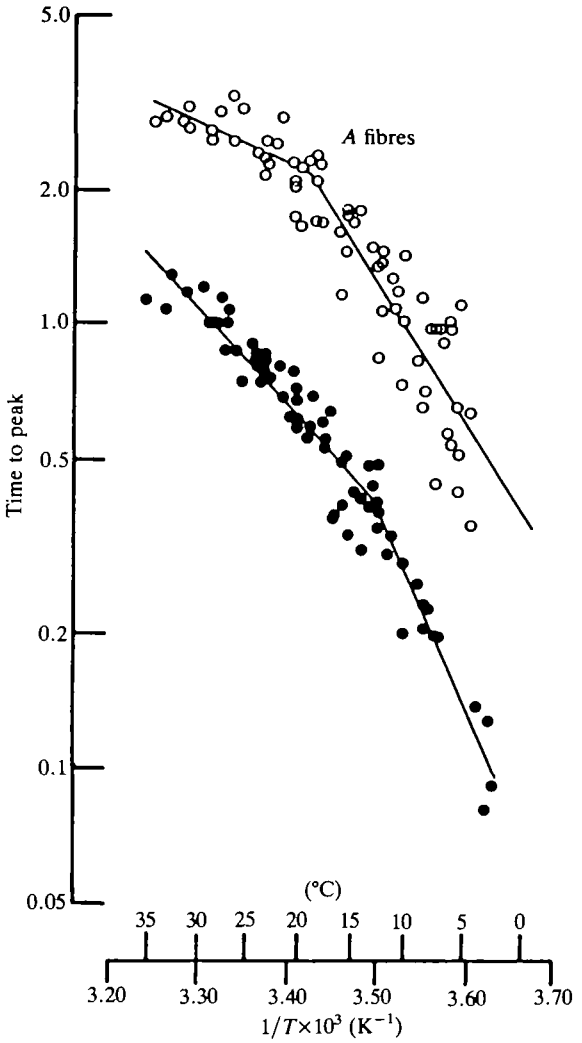

Fig. 5. Comparison of Arrhenius plots for the *A* fibre action potentials for nerves from warm-acclimated (●) and cold-acclimated (○) fish (N=4 in each group). Values are expressed as a fraction of that measured at the acclimation temperature. The data were best fitted by two separate linear regressions with a break point at 13.1 °C (P<0.01) for warm-acclimated and 19.3 °C (P<0.01) for cold-acclimated nerves. The apparent activation energy (E_a) values at temperatures above and below the break were 42.0±2.16 and 86.9±4.55 kJ mol^{-1} for the warm-acclimated and 18.0±5.45 and 58.8±4.08 kJ mol^{-1}, respectively, for the cold-acclimated fish.

the warm-acclimated and 13.2±3.6 kJ mol^{-1} (N=4) for the cold-acclimated fish. However, those for conduction below the break were greater for nerves from warm- than from cold-acclimated fish: E_a 49.7±3.78 and 41.2±2.65 kJ mol^{-1}, respectively (N=4). Similar analyses for the *A* fibre data give an E_a of 42.04±2.16 kJ mol^{-1} (N=4) for the warm-acclimated and 18.02±5.45 kJ mol^{-1}

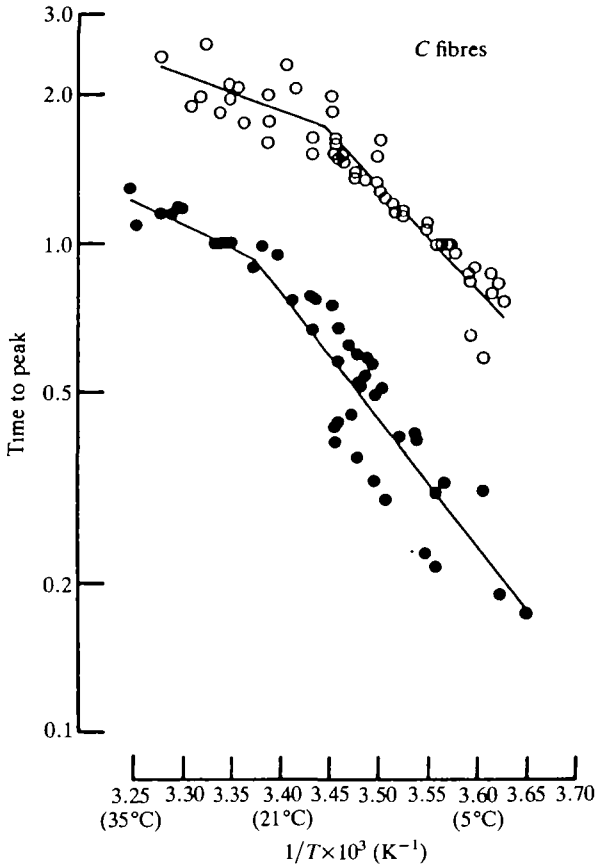

Fig. 6. The effect of temperature on the conduction velocity of the *C* fibre action potentials recorded from nerves from cold-acclimated (○) and warm-acclimated (●) fish (N=4 in each group). The data for both groups were best fitted by two linear regressions. The split or break-point was at 23.6°C ($0.01<P<0.05$) for warm-acclimated nerves with apparent activation energies (E_a) for the conduction process of 49.7±3.78 and 18.3±8.33 kJ mol^{-1} below and above this temperature, respectively. The break point for cold-acclimated nerves was at 17.4°C ($P<0.01$) with E_a values of 41.2±2.65 and 13.2±3.63 kJ mol^{-1} below and above this temperature, respectively.

(N=4) for the cold-acclimated nerves above their break points of 13.1 and 19.3°C, respectively. Below the break point (low temperatures), the E_a for conduction in the warm-acclimated fish was much greater than that for cold-acclimated material: E_a 86.9±4.55 and 58.8±4.08 kJ mol^{-1}, respectively.

Membrane fluidity

The steady-state fluorescence polarizations of DPH incorporated into myelin and synaptosomal fractions of carp brain were measured at 8, 18 and 28°C. The results of the pooled material from preparations from 8°C- and 28°C-acclimated fish are shown in Fig. 7. Myelin-rich fractions were more ordered than the

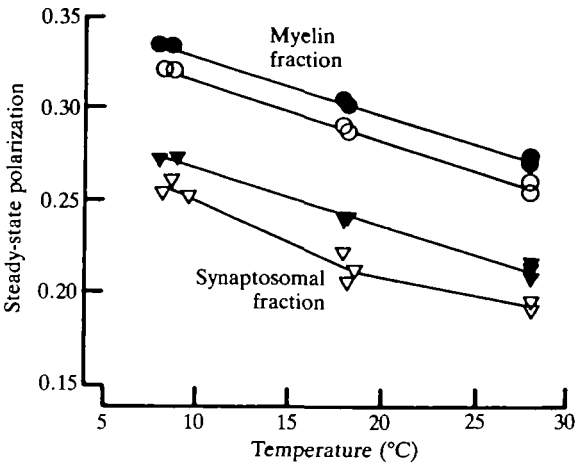

Fig. 7. Steady-state polarization of DPH-labelled membrane fractions as a function of temperature and prior acclimation temperature. ●,▼, warm-acclimated fish; ○,▽, cold-acclimated fish.

synaptosomal fraction, as is generally found (Cossins, 1983), and increasing the temperature of each reduced the order (i.e. increased fluidity) by a similar amount. The effect of prior temperature acclimation on the two fractions appeared as a translation of the curves (Fig. 7), with a partial compensation for the effect of temperature on fluidity, as seen in other preparations (Cossins, 1983). Thus, acclimating fish at 8°C, as compared to 28°C, caused an increase in the fluidity of the two membrane fractions. The biggest effect was seen in the synaptosomal fraction measured at 18°C, in which the polarization values of the 8°C-acclimated membranes are equal to those of 28°C-acclimated membranes, measured at 28°C.

Lipid composition

The fatty acid composition of the major phospholipids for the myelin and synaptosomal fractions of brain from warm- and cold-acclimated fish are listed in Table 1. No obvious differences were apparent in the proportions of individual phospholipid fatty acids in either fraction from warm- and cold-acclimated fish.

Discussion

The conduction velocities of the myelinated (*A*) and unmyelinated (*C*) components of the compound AP were affected by temperature in a consistent way that was apparently related to the acclimation temperature of the donor animals. The compound AP is a complex phenomenon. Its amplitude at any instant represents the algebraic sum of current flow in the contributing axons and changes in the amplitude or conformation of a component of the AP may reflect changes in the number of individual conducting fibres, their conduction velocity

Table 1. *Phospholipid fatty acid composition of synaptosomal and myelin fractions of carp brain*

	Synaptosomal		Myelin	
	Cold	Warm	Cold	Warm
C14:0	1.00	Trace	1.01	0.87
C16:0	13.33	12.09	15.95	14.97
C16:1ω7	2.98	2.92	10.21	9.32
C16:1ω9	1.14	1.30	5.87	6.22
C16:2	0.27			
C16:3ω3			1.15	0.97
C16:4	0.81		3.20	4.21
C18:0	9.08	10.95	5.47	6.61
C18:1ω7	6.42	5.13	2.81	2.30
C18:1ω9	9.91	14.38	26.43	29.92
C18:2ω6	1.82	0.57	2.02	1.35
C20:1ω9	1.24	Trace	2.26	1.68
C20:3ω6	0.69		1.10	0.74
C20:4ω6	3.90	4.46	2.73	3.10
C20:5ω3	4.27	Trace	2.07	0.80
C22:6ω3	41.11	48.13	12.46*	10.36*
C24:1ω9			5.14*	6.47*

* Estimated value.

Data are presented as the percentage of the total chemical identities assayed. Each result is the mean of three individual measurements of material pooled from four brains (in each scan the sum of the percentages identified was 99.99 %).

and their synchronisation. Changes in the duration of the AP will also have an effect. It seems likely, however, that with the present recording configuration the latter is not important. The temperature dependence of the conduction velocity of the *A* and *C* components of the AP displayed a discontinuity. The temperature sensitivity of the conduction process above and below the transition temperature was dependent on the acclimation temperature of the animal. In these circumstances conduction velocity is most likely to reflect the amplitude of the inward, sodium current. This will be determined by the gating characteristics of the channel, assuming no changes in ionic balance occur.

Several experiments have been reported showing that a temperature-dependent transition exists for the conductance of the sodium channel in mammalian (rabbit, *Oryctolagus cuniculus*) and amphibian (*Rana pipiens*, *Rana catesbeiana* and *Rana temporaria*) nerve (Chiu *et al.* 1979; Schwartz, 1979). Furthermore, the temperature at which the transition occurred was higher for rabbit than for frog nerve. The break or discontinuity in the Arrhenius plot of conductance parameters may reflect a conformational change in the protein of the ion channel. Alternatively, it

may be a consequence of a lipid phase transition which could modify the functioning of the channels. The concept of lipid composition and physical state modulating the activity of membrane proteins is well documented (Deuticke and Haest, 1987). One interpretation of the results of the present experiments is that the channel protein, its boundary lipid or the lipid bilayer as a whole undergoes a phase transition. Thermal acclimation of the animal to warm or cold environments would alter the active temperature sensitivity of the conduction process in a compensatory manner.

The fluorescent polarization results of DPH-labelled brain membrane fractions were consistent with partial homeoviscous adaptation. This raises the important question of whether there is a causal relationship between the observed changes in the membrane fluidity and the electrophysiological properties of the vagus. In the present study it was not feasible to collect sufficient peripheral nerve for fluidity determination or lipid analyses. Instead, we analysed synaptosome and myelin fractions from the brain and assumed that comparable changes also took place in peripheral nerve. In garfish, the unmyelinated olfactory nerves and myelinated trigeminal nerves undergo similar changes in lipid composition (Friedman *et al.* 1986).

The steady-state fluorescence polarization of DPH in membranes provides an index of order or fluidity (Jahnig, 1979; Van Blitterswijk *et al.* 1981; Van der Meer *et al.* 1986). The values reported here are entirely consistent with the findings of others (Cossins, 1983; Cossins and Macdonald, 1989) and are evidence that brain membranes undergo a partial compensation for the ordering effect of reduced temperature. Attempting to relate these fluidity changes to the fatty acid composition of the same membrane fractions shows that the fatty acids in the myelin fraction cannot account for their greater degree of order than those in the synaptosomal membranes (Table 1). Similarly, the changes in fluidity accompanying temperature acclimation do not relate to the changes in the fatty acid composition in any obvious way. In view of the incomplete analysis (cholesterol and membrane protein contributions were not determined because of low sample size) this is perhaps not surprising.

One theory of acclimation to a reduced temperature suggests that increased amounts of unsaturated fatty acids in specific membrane phospholipids should compensate for the increase in order and safeguard the normal functioning of specific membrane proteins, such as ion channels and other components which might determine conduction rates. The partial compensation evident in the polarization data, and the limited changes in the fatty acid composition (which are, of course, averages for a suspension of membranes), imply a very restricted role for phospholipids. The acclimatory compensation shown in the conduction velocity of both *A* and *C* fibres is much greater than that seen in membrane fluidity.

Taken together, the results from this study emphasise the difficulties using simple models to explain the mechanisms of temperature adaptation in excitable membranes. Alterations in the physical state of the membrane lipid may be

responsible for the changes in activation energy. Changes in the composition occurring with acclimation may underlie the shift seen between warm and cold material. The differences in ion channel density, membrane structure and lipid surround between the node of Ranvier of the myelinated and unmyelinated nerve axons may underlie the differences in thermal acclimation. Various other sites of, and mechanisms for, the temperature adaptation of nerve are, of course, possible. For example, changes in axon morphology or state of myelination will affect conduction velocity. Indeed, a change in axon diameter spectrum of thickness of myelin fibres in the optic nerve of the goldfish (*Carassius auratus*) has been reported by Matheson and Roots (1988). They found a shift towards the occurrence of larger fibres in warm-acclimated compared with those of their 5°C-acclimated counterparts. If this behaviour is shared by peripheral axons it could explain the higher conduction velocities seen here at any given temperature by nerves from cold-acclimated compared with warm-acclimated fish.

Alternatively, changes in ion channel density may be responsible. It has been calculated that the sodium channel density in small unmyelinated nerves is considerably less than optimal for maximal conduction velocity (Hodgkin, 1975). It was argued that this low density is necessary to reduce ionic exchange during the action potential and thus minimise the energy expenditure of recovery pump flux. Increased sodium channel density may be an adaptation for the maintenance of impulse conduction velocity. A concurrent upward adjustment of ion pump density would be expected to maintain the channel/pump flux ratio at unity, as has been described for polar fishes (Hochachka, 1988). These alterations might be distinguished by further, direct investigation.

These results show that in the freshwater carp, the temperature dependence of the conduction velocity of the vagus nerve is affected by the prior temperature experienced by the animal. A partial compensation occurs, that is, the effects of acute temperature changes are diminished and, accordingly, such changes could well be adaptive in nature.

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