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Differential Effects of Hypoosmotic Hyponatric Swelling on A and C Fibers

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The differential effects of exposure to a moderately hypoosmotic hyponatric solution (0.35 isoosmotic, Na^+ 36 mmol/l) on conduction in myelinated (A) and unmyelinated (C) axons were studied *in vitro* on compound action potentials of rabbit vagus nerves in which the perineurial sheath had remained undisturbed. Controls were incubated at 37° C in isoosmotic isonatric solution for 5 h (Group 1a, n = 7) or 7 h (Group 3, n = 3). Other controls were incubated in isoosmotic isonatric solution for 2 h followed by 3 h in isoosmotic hyponatric (Na^+ 36 mmol/l) solution (Group 1b, n = 6); experimental nerves were incubated in isoosmotic isonatric solution for 2 h followed by 3 h in hypoosmotic hyponatric solution (Group 2, n = 7) and, to study recovery, a further 2 h in isoosmotic isonatric solution (Group 4, n = 8). In Group 1b, isoosmotic hyponatric exposure approximately doubled the latency of the A-component (A-CAP) and decreased the A-CAP amplitude to 44 ± 8% of control; the amplitude of the C-component decreased to 65 ± 15% of control. Hypoosmotic hyponatric exposure increased the latency of the A-CAP by 82 ± 10% (mean ± SE, $P < 0.001$) and extinguished A-CAP within 20 min, whereas the latency increase of the C-component (C-CAP) was more than twice as great and extinction slower and often incomplete; neural wet weight increased 34 ± 4% and neural sodium and potassium contents decreased 55 and 42%, respectively. Recovery in isoosmotic isonatric solution (Group 4) was absent or very small in the case of A-CAP, as regards latency and amplitude but was complete for C-CAP amplitude. Neural wet weights and sodium content also recovered fully, but neural potassium content recovered only about 45%. Electron microscopy revealed hypoosmotic hyponatric structural damage to the larger myelin sheaths; the axons themselves were unaffected. It is concluded that it is probably inadvisable to attempt selective conduction block of sensory C-fibers by application of hypoosmotic solutions to peripheral nerves. (Key words: Nerve: block, differential. Ions: Sodium, potassium.)

TEMPORARY OR PERMANENT differential conduction block of unmyelinated C axons sparing large myelinated A fibers is often highly desirable in the treatment of acute or chronic pain. Selective destruction of unmyelinated C axons after exposure to solutions of marked hypoosmolarity has been reported previously.[1,2] Moderately hypoosmotic solutions are known to produce reversible C fiber conduction block,[3]§ and it has been shown that osmotic fragility of axons is similar to that of erythrocytes.[3,4] We report the differential effects of moderate hypoosmotic hyponatric exposure on conduction in A and C axons of rabbit vagus nerves with an intact perineurium. A selectively destructive effect on A axons was observed.

Methods

Male New Zealand rabbits weighing 2.5–3.0 kg were sacrificed by air embolus and perfused with lactated Ringer's solution (Travenol Laboratories) via the left ventricle. Cervical vagus nerves were carefully excised and weighed immediately on a Mettler H64 precision balance to the nearest 0.01 mg. Within 50 s, the nerves were submerged in isoosmotic isonatric Ringer's bicarbonate solution containing (mmol/l) NaCl 112.5, KCl 4, NaH_{CO_3} 24, $CaCl_2$ 2.2, $MgSO_4$ 0.8, and glucose 20, equilibrated with 5% CO_2–95% O_2 at 36–38° C. The total osmolarity measured with a Wescor osmometer was 270–290 mmosm/l. The high concentration of glucose (360 mg/dl) is necessary for optimal preservation of function by mammalian large axons *in vitro*.[5,6] Each nerve was weighed after 1 h in the isoosmotic isonatric solution and then fixed to an array of platinum electrodes by droplets of agar, the distal end to the recording and the proximal end to the stimulating electrodes. This assured orthodromic conduction in the efferent fibers. The assembly then was immersed in a closed chamber and further incubation performed. For electrophysiologic observations, the array was raised out of the solution without opening the chamber.

Direct current from a capacity isolation unit was delivered to the stimulating electrodes (Anapulse stimulator—model 301 and model 305 battery, WP-Instruments Inc.). Stimulus duration was 0.1 ms for A-component and 1 ms for C-component of the recorded compound action potentials. After determining the threshold stimulus strength for each fiber group, supramaximal stimuli were applied (1 V and 100 V, respectively). The compound action potentials were amplified and displayed on a Tek-

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tronix 202-1-model B-oscilloscope. Photographic records were made with a Polaroid® camera. During subsequent treatments, the nerves were raised out of the solution and stimulated in the closed incubation chamber every 5–60 min. The amplitude of the fast (A) component of the action potential was measured from base to peak, that of the slow component (C) from positive peak to negative peak. The latency of each component was measured from the stimulus onset to the first peak of the potential.

After installation on the electrodes, the nerves were maintained for a further hour in the isoosmotic isonatric solution, and the three last sets of measurements during this hour were averaged to give baseline (100%) amplitude and latency.

Thirty-one nerves were studied in four groups. Nerves of Group 1a (n = 7) were maintained in isoosmotic isonatric solution for 5 h. Nerves of Group 1b (n = 6) were maintained in isoosmotic isonatric solution for 2 h, followed by 3 h in isoosmotic hyponatric solution (NaCl 12 mmol/l, total Na 36 mmol/l—other constituents being the same as in the isoosmotic isonatric solution; enough choline chloride was added to render the solution isoosmotic). Nerves of Group 2 (n = 7) were maintained in isoosmotic isonatric solution for 2 h followed by 3 h in hypoosmotic hyponatric solution (0.35 isoosmotic, Na 36 mmol/l; the required hypoosmolarity was obtained by restricting the NaCl concentration in this solution to 12 mmol/l; the other constituents were the same as in the isoosmotic solution). At the end of the 5 h, incubations the nerves of Group 1a and 2 were weighed, desheathed, and dried overnight at 100° C. The dried tissue was weighed and then dissolved in 1 ml nitric acid in a crucible and heated until dry. The residue, representing the nerve core, was dissolved in distilled water and its Na^+ and K^+ content expressed as mmol/kg dried nerve tissue, determined by flame photometer (model 143, Instrumentation Laboratory Inc.). Nerves in Group 3 (n = 3) and 4 (n = 8) were incubated similarly to those in Groups 1 and 2 but then were exposed to isoosmotic isonatric solutions for a further 2 h to test reversibility of the effects observed in Group 2. Statistical significance of differences between control and treatment groups was evaluated by two tailed *t* test for unpaired observations; paired *t* test was used for the weight changes within each group.

Light and electron microscopic studies were performed in four nerves, two Group 1a controls and two Group 2 hypoosmotic hyponatric exposures. Specimens from these nerves were fixed in 5% glutaraldehyde made up in the same hypoosmotic or isoosmotic solution to which each nerve had been exposed experimentally. The specimens were postfixed in 1% buffered osmium tetroxide, dehydrated, and embedded in epoxy Epon. Semithin (1 μm) sections were stained with methylene blue for light microscopy and appropriate blocks selected for electron microscopy. Thin sections for electron microscopy were stained with uranyl acetate and lead hydroxide and examined in an AEI EM6-B electron microscope.

Results

Compound action potentials from a representative nerve of Group 1b (n = 6) are presented in figure 1. This group tested the degree of preservation of function in the presence of hyponatricity but in the absence of hypoosmolarity. In this group, the decrease in amplitude of the A and C potentials was, respectively, to 44 ± 5 and 65 ± 11% of control (mean ± SE) and the increase in latency to 182 ± 7 and 144 ± 7% of control (table 1). With one exception, these changes were obviously different from those effected over the same period of time in nerves treated with a solution that was both hyponatric and hypoosmotic (Groups 2 and 4, fig. 2 and table 1). In hypoosmotic hyponatric solution (Group 2 and Group 4, n = 15) the A component of the compound action potential was extinguished within 20 min and the C com-

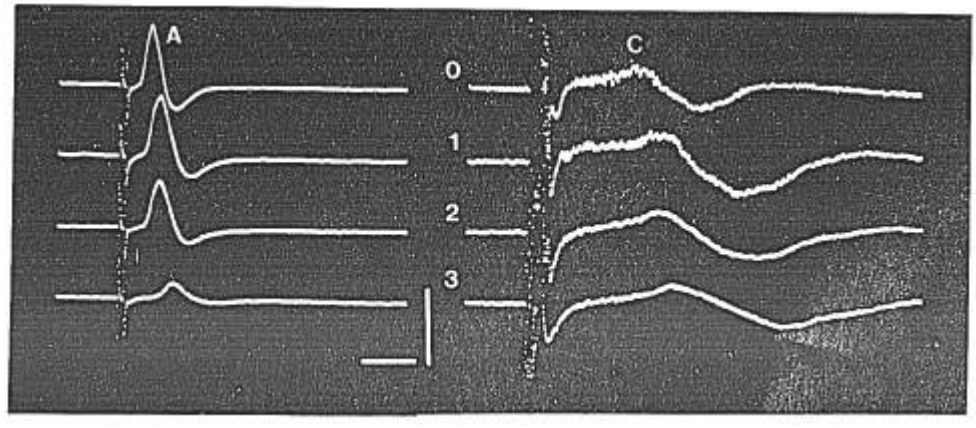

FIG. 1. A and C compound action potentials from a nerve of Group 1b before exposure to isoosmotic hyponatric solution (0) and after 1, 2, and 3 h in the solution. Compare with Figs. 2 and 3, where the ambient solution was hypoosmotic as well as hyponatric. Scale bars: 1 mV, 2 ms for component A, 0.5 mV, 10 ms for component C.

TABLE 1. Amplitude and Latency of A and C Components of Potentials Elicited from Nerves in Isoosmotic (Groups 1 and 3) and Hypoosmotic (Groups 2 and 4) Incubations*

Group	Group 1a		Group 1b		Group 2/4		3		4	
Solution and Exposure time	Isoosmotic Isonatric 5 h		Isoosmotic Isonatric 2 h; Isoosmotic Hyponatric 3 h		Isoosmotic Isonatric 2 h; Hypoosmotic Hyponatric 3 h		Isoosmotic Isonatric 7 h		Isoosmotic Isonatric 2 h; Hypoosmotic Hyponatric 3 h; Isoosmotic Isonatric 2 h	
Component	A	C	A	C	A	C	A	C	A	C
Amplitude	87 ± 4	93 ± 4	44 ± 4	65 ± 11	0 ± 0	16 ± 5	87 ± 3	93 ± 7	19 ± 12	106 ± 6
Latency	103 ± 2	97 ± 2	182 ± 7	144 ± 7	182 ± 10†	430 ± 50†	100 ± 0	98 ± 2	241 ± 12	119 ± 4
n	7/3		6		7/8		3		8	

* Values are expressed as percentage of preincubation value (mean ± SEM).
† Preextinction latency.

ponent averaged 16% of baseline amplitude after 3 h in the solution (fig. 3). The decrease in amplitude was accompanied by an increase in latency, the preextinction increase in latency of the C component being more than twice as great as that of the A component (fig. 4). These effects differed significantly from those in Group 1b ($P < 0.001$). The noted exception concerns the increase in latency of the A component before extinction, which was the same irrespective of the presence or absence of hypoosmolarity. While in hypoosmotic hyponatric solution (Groups 2 plus 4), the extinguished A fibers remained nonexcitable even to increased (×100) voltage stimulation. In contrast, the C component remained at least partly observable throughout the hypoosmotic hyponatric exposure in three nerves. In addition, in seven of the 12 nerves where the C component was temporarily extinguished partial recovery began before the end of the hypoosmotic hyponatric exposure (table 2).

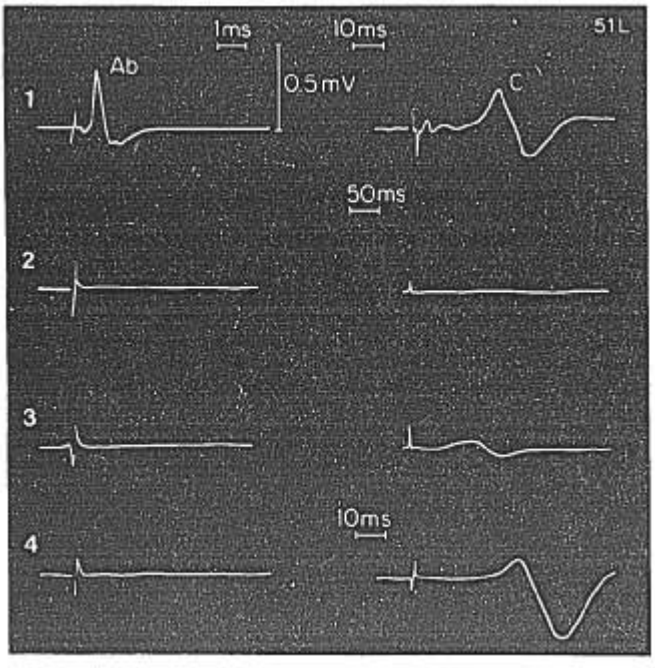

FIG. 2. A and C compound action potentials from a representative nerve of Group IV during hypoosmotic hyponatric exposure and recovery in isoosmotic isonatric solution (retouched). 1 = control; 2 and 3 = 60 and 180 min in hypoosmotic hyponatric solution; 4 = 120 min isoosmotic isonatric recovery. Note change of time scale for component C in 2 and 3. Traces 1–3 are also representative of Group 2.

The reversibility of the hypoosmotic hyponatric effects was studied in Group 4 by transferring the nerves into isoosmotic isonatric solution (containing glucose 20 mmol/l) for 2 h, or in three cases, for 16 h. The amplitude of the C component recovered completely (fig. 3, table 1), the latency almost completely (fig. 4, table 1). However, the amplitude and latency of the A component recovered very little (figs. 3 and 4, table 1) and in four nerves remained nonexcitable though raised (×100) voltage stimulation was applied (table 2) ($P < 0.001$). No recovery of the A component was seen in two of the three nerves observed for 16 h.

The weights of the nerves and the sodium and potassium contents of the desheathed nerve cores are presented in figure 5. A large increase in weight of nerves was observed in Group 2 ($P < 0.001$), a small increase in Group 1a ($P < 0.01$), but no change in Group 3. Group 4 showed a small but significant net loss of weight ($P < 0.05$).

Compared with Group 1a, Group 2 nerves analyzed after 3 h in hypoosmotic hyponatric solution had lost almost half of their sodium and potassium ($P < 0.001$). Group 4 nerves analyzed after 2 h of recovery apparently regained the lost sodium completely but only about half of the lost potassium ($P < 0.001$).

Morphologic examination of control nerves incubated

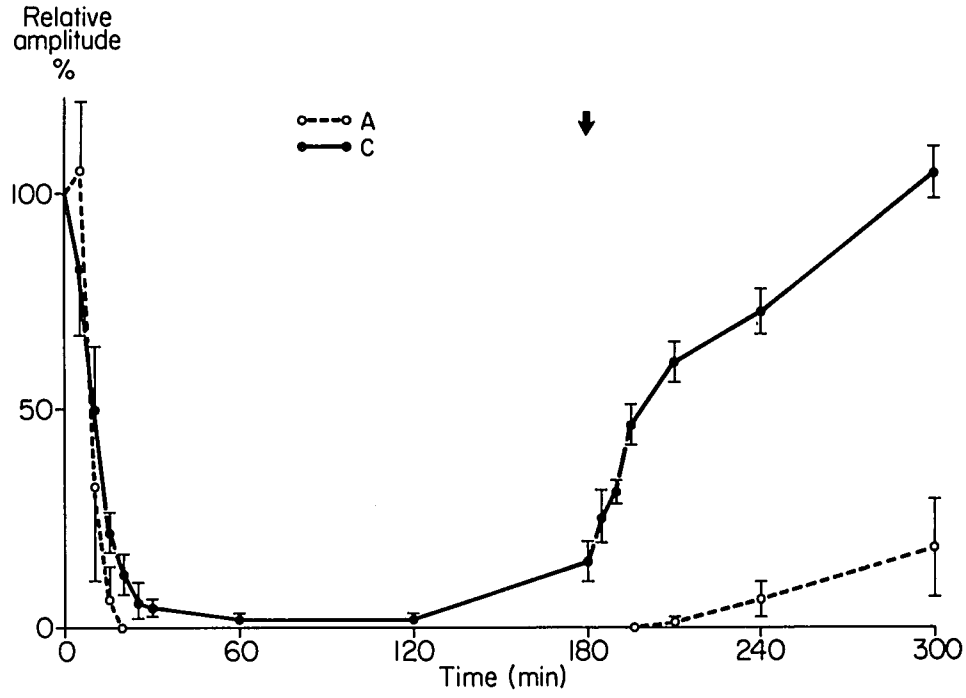

FIG. 3. Relative amplitude of A and C compound action potentials during hypoosmotic hyponatric exposure and (at arrow) recovery (bars = SEM; Groups 2 and 4).

in isoosmotic isonatric solutions for 5 h (Group 1a) showed normal myelinated and unmyelinated axons. In transverse sections both myelinated and unmyelinated axons were present, and the thickness of the myelin sheath appeared to be directly proportional to the diameter of its axon (fig. 6*A*). Nerves exposed to hypoosmotic hyponatric solutions (Group 2) showed good preservation of both myelinated and unmyelinated axis cylinders. However, the myelin sheath of some thick fibers showed extensive disruption (fig. 6*B*).

Discussion

The slower extinction of the C fiber component during hypoosmotic hyponatric exposure and its more extensive recovery than the A component (Group 4) demonstrate that, in a nerve with intact perineurium, unmyelinated axons withstand lowering of the external sodium ion concentration and accompanying cellular osmotic swelling much better than myelinated axons. Although the relative roles of low sodium and low osmolarity cannot be eval-

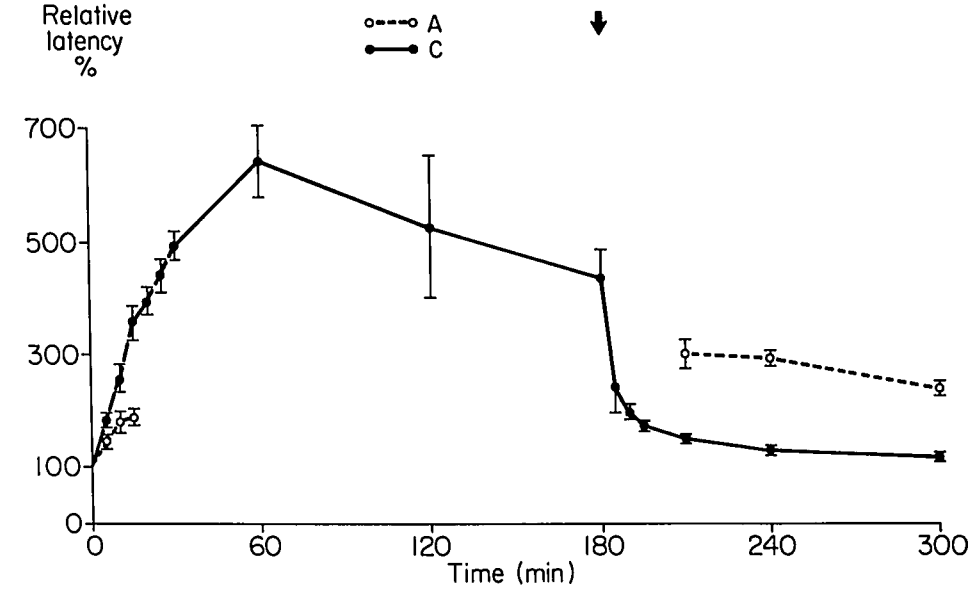

FIG. 4. Relative latency of A and C components during hypoosmotic hyponatric exposure and (at arrow) recovery (bars = SEM; Groups 2 and 4).

TABLE 2. Number of Nerves Partially Retaining A and C Potentials at Indicated Times during Hypoosmotic Exposure in Groups 2 and 4 (n = 15) and upon Return to Isoomotic Solution in Group 4 (n = 8)

	Group 2 plus 4									Group 4					
Time (min)	5	10	15	20	25	30	60	120	180	185	190	195	210	240	300
Component															
A	14	7	3	0	0	0	0	0	0	0	0	0	2	3	4
C	15	14	13	9	6	6	3	3	10	6	8	8	8	8	8

uated from the data, it is likely that hypoosmolarity played some part in the extinctions, since low sodium in the presence of isoosmolarity (Group 1b) did not produce extinction. The observations on A fibers in Group 1b may be compared with those of Nathan and Sears[7] on cat spinal rootlets where 15 min exposure to a solution containing 20% of the normal sodium content had no visible effect, whereas 15 min in 12.5% of the normal sodium content caused the amplitude to decrease by one-third. The observations on C fibers in Group 1b may be compared with those of Colquhoun and Ritchie[8] on rabbit cervical vagus nerve C fibers; these workers noted that after ½ to ¾ h exposure to an ambient sodium concentration 20% of normal, the amplitude of the C potential was still 75% of control. As regards the role of failure of the sodium "pump," the extent of the Na dependence of Na-K dependent ATPase in intact nerve fibers is not known. However the above-mentioned ability of mammalian nerve fibers to preserve excitability in a low external Na^+ environment suggests that the Na^+ dependent pumps were probably still functioning to some extent.

The observed differential is the reverse of that reported in spinal rootlets by Jewett *et al.* and by King *et al.*[1,2] The principal structural difference between peripheral nerve and spinal rootlets resides in the investing tissue: unlike a rootlet, a peripheral nerve is surrounded by perineurium. Perineurium consists of one or more concentric layers of flattened cells, each layer being bounded on both the epineurial and endoneurial surfaces by a basal lamina.[9,10] In the cervical portion of rabbit vagus, which is a single fasciculus, the perineurium has typically three or four layers of cells, and there are thus at least six concentric basal laminae, whereas in spinal rootlets there is only one.[11] This structural difference may account at least in part for the difference in the effects of hypoosmotic hyponatric stress in spinal rootlets and vagus nerve. It is relevant that in renal tubules the basal lamina has been identified experimentally as the seat of the resistance of renal tubular epithelium to hypoosmotic swelling.[12] It was interesting to note that in Group 2 of the present study, the swollen vagus nerves became strikingly turgid and stiff. The flat cells of each perineurial layer are joined to each other by tight junctions[9,10] that resist the passage of electrolytes such as sodium and potassium.[13] Hypoos-

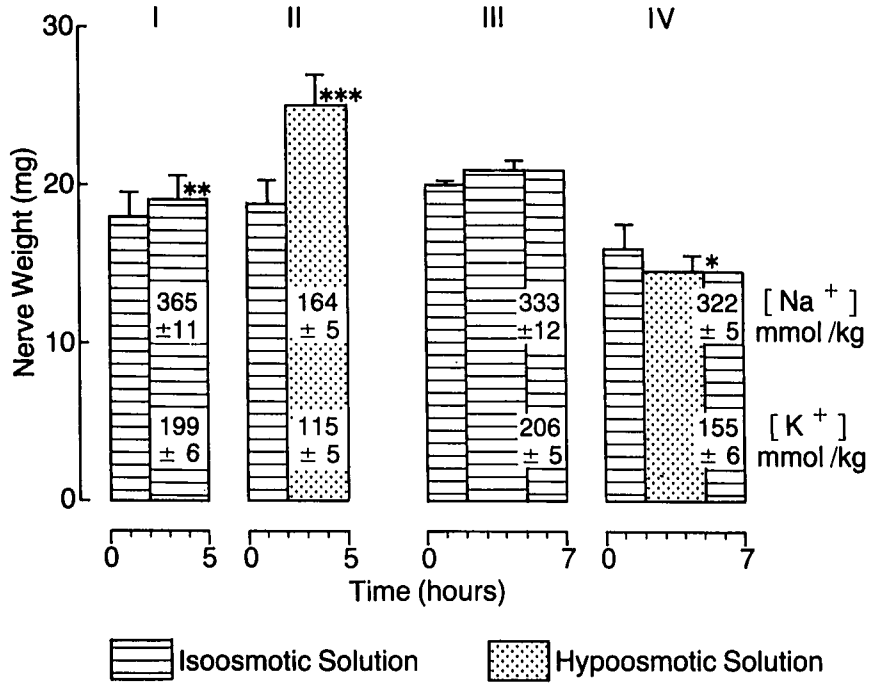

FIG. 5. Nerve weights (wet) at the start and at the end of experiments (mean ± SEM). Exposures: I = 5 h isoosmotic, II = 2 h isoosmotic isonatric + 3 h hypoosmotic hyponatric, III = 7 h isoosmotic isonatric, IV = 2 h isoosmotic + 3 h hypoosmotic hyponatric + 2 h isoosmotic isonatric. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. The numbers within the bars indicate the Na^+ and K^+ contents of the nerves at the end of the experiments (mean ± SEM).

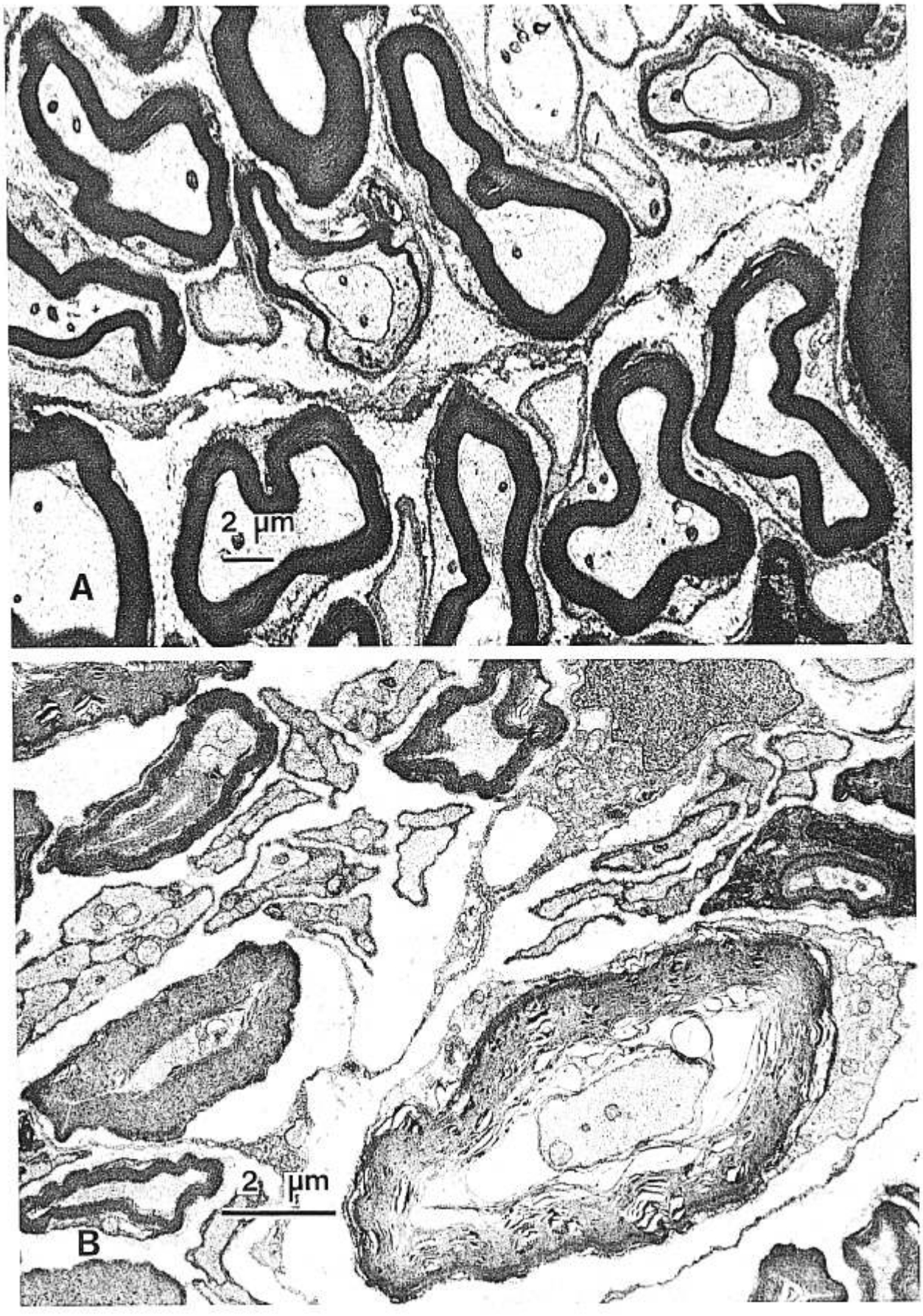

FIG. 6*A* (*top*). Electron micrograph of transverse section of a control nerve (Group 1a) showing both myelinated and unmyelinated axons. *B* (*bottom*). Electron micrograph of transverse section of a nerve incubated in hypoosmotic hyponatric solution (Group 2). Axons, both myelinated and unmyelinated, appear to be well preserved, but the myelin sheath of one large axon shows disruption of its inner layers.

motic hyponatric exposure, besides depleting the transmembrane gradients of sodium and potassium, therefore will result in a general hypoosmotic swelling of the tissues internal to the perineurium and cause an increase of pressure on the axons comparable to some extent to that produced by inflation of an overlying tourniquet in a limb. Compression by a tourniquet is known to have a selective depressant action on conduction in fast conducting fibers[14,15] and, at an inflation pressure of 1,000 torr, to severely distort the myelin sheath while leaving unmyelinated fibers morphologically intact.[16] Thus, the known functional and structural resistance of unmyelinated axons to external hydrostatic pressure can account at least partly for the resistance of the C component to extinction in our experiments and for its greater ability to recover.

The observed changes in weight correlate quite well with the measured changes in electrolyte content of the nerves (fig. 5). Movement of water into the nerves caused an increase in weight during hypoosmotic hyponatric incubation (Group 2). A net loss of electrolytes—mainly potassium—and accompanying water at the end of recovery may account for the small residual weight loss. As shown in figure 5, hypoosmotic hyponatric exposure (Group 2) caused the nerves to lose roughly half their sodium and potassium. The period of recovery in isoosmotic isonatric solution (containing glucose 20 mmol/l) (Group 4) caused the sodium level to return almost to normal, whereas in the case of potassium, only about half of the loss was recovered. The difference in the recovery of neural sodium and potassium levels is understandable in the light of the different concentrations of these ions in the extracellular and intracellular compartments and the respective volumes of these compartments. In cervical vagus nerve of rabbits, these two compartments are known to be of approximately equal volume.[17] The large neural loss of sodium during hypoosmotic hyponatric exposure therefore must have been overwhelmingly from the extracellular compartment and relatively little from injured cells and could readily recover on return to isoosmotic isonatric conditions. The large potassium loss, however, must have been mainly from the intracellular compartment and would recover presumably only to the extent that axonal function recovered. Since recovery was restricted to C axons, which recovered almost completely, and since the volume of C axons in cervical vagus nerve is almost half the total axonal volume,[17] the extent of the observed recovery of potassium content correlates well with almost complete recovery of the compound action potentials limited entirely to the C component (figs. 2, 3, and 4). Microscopic studies demonstrate structural effects of the same type as previously produced with distilled water by Robertson[18] in which there was disruption of the myelin sheath, and unmyelinated axons were relatively unaffected.

The earlier idea that hypoosmolarity might be a useful characteristic of solutions used for peripheral conduction block of nociception[1-3] is not supported by the results of the present study. Unfortunately, the differential effect of external hyponatric hypoosmolarity on large and small axons in sheathed nerve seems to be such as to favor a selective preservation of function in small axons of the type that subserve nociception.

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