ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6): 481 - 489
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Original Research

Comparisons of somatic action potentials from dispersed and intact rat nodose ganglia using patch-clamp technique[1]

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KEY WORDS afferent pathways; nodose ganglion; action potentials; neurons; vagus nerve; patch-clamp techniques

ABSTRACT

AIM: To differentiate the electrophysiological characteristics of somatic action potentials (AP) from isolated Neo and Juv nodose sensory neurons (NSN) and those from slices of intact Juv and adult rat nodose ganglia. **METHODS**: For isolated cell recordings nodose ganglia from 3 – 8 d old Neo and 4 weeks old Juv rats were dissociated using trypsin and collagenase, respectively. Nodose ganglia slices with attached vagus were prepared using a sequential treatment of collagenase and trypsin for both Juv and adult rats. Conduction velocity (CV) was collected by vagal stimulation. Whole-cell patch was applied for somatic AP recordings. **RESULTS**: (1) 281 NSN from both isolated cells and nodose slices were studied. Across all age groups, there was no difference observed among either C- or A-types. The difference between C- and A-type was significant. (2) Neurons exhibiting AP with prominent repolarization hump, broader APD_{50} (> 2.0 ms), upstroke velocity at the point of APD_{50} ($UV_{APD_{50}}$) and downstroke velocity at the point of APD_{50} ($DV_{APD_{50}}$) below 100 $V \cdot s^{-1}$ and 50 $V \cdot s^{-1}$ were classified as C-type ($n = 222$). Those without a hump, brief APD_{50} (< 1.0 ms), and $UV_{APD_{50}}$ and $DV_{APD_{50}}$ greater than 200 $V \cdot s^{-1}$ and 100 $V \cdot s^{-1}$ were classified as A-type ($n = 59$). (3) With slices, except for hump, APD, UV, and DV, significant differences were also observed (C- *vs* A-type) in CV from both Juv (0.56 ± 0.15 *vs* 15.6 $m \cdot s^{-1}$) and adult (0.9 ± 0.4 *vs* 14.5 ± 1.0 $m \cdot s^{-1}$) nodose slices, discharge rate (single or burst *vs* repetitive), and frequency follow (< 20 Hz *vs* > 100 Hz). (4) Phase analysis showed that C-type had higher AP firing threshold, and lower total in- and outward peak currents than A-type. **CONCLUSION**: C- and A-type AP from isolated NSN of Neo or Juv rats exhibited electrophysiological characteristics that closely followed those of AP recorded in nodose slices. Therefore, isolated NSN can effectively serve as an experimental surrogate for electrophysiological studies of NSN requiring prior identification of afferent fiber type. Features of the AP waveform such as the repolarization hump, APD_{50}, and UV are strongly correlated with CV and are therefore reliable measures for classifying isolated NSN as either C- or A-type. It is most important that the nodose slice enables us to study the AP and ion channel characteristics on identified NSN by afferent fiber CV with adult animals.

INTRODUCTION

Nodose sensory neurons (NSN) support afferent projections between a wide range of visceral organ systems and the central nervous system[1-3]. The majority of cardiovascular afferent fibers with cell bodies in the nodose ganglia travel along the vagus nerve and project onto second order neurons in the medial nucleus of the solitary tract. Therefore, NSN play a critically important role in the neural control of cardiovascular function[4,5]. Previous studies[6-9] have revealed two general populations of NSN based upon action potential discharge characteristics. One group, representing 90 % of the total population of NSN exhibited broad somatic action potentials (AP) with a prominent "hump" in

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Received 2001-10-17 Accepted 2002-02-08

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
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membrane voltage over the course of AP repolarization. Often classified as C-type, these neurons tended to discharge with a single or short burst of AP in response to step depolarizations. The remaining population of cells exhibited a brief AP duration (APD) and a smooth repolarization. Classified as A-type, these cells discharged repetitively in response to step depolarizations.

The enzymatic dispersion of sensory ganglia has made possible the isolation of individual NSN for study using higher resolution electrophysiological techniques under carefully controlled experimental conditions. Unfortunately, the chemical and mechanical trauma associated with this technique transects all axons, leaving no direct measure of afferent fiber type. Recently, our lab developed a technique[10] that made possible the patch clamp recording of adult neurons from intact nodose ganglia with an attached vagus nerve. The preparation made possible the detailed electrophysiological study of somatic AP properties from neurons that had been unambiguously classified as C- or A-type based upon conduction velocity (CV) measurements. In the present study somatic AP recorded from isolated Neo and Juv NSN were compared with those recorded from intact Juv and adult nodose ganglia. We demonstrate here that the discharging properties of AP can be conducted on identified C- or A-type NSN with adult animals. An isolated cell preparation can serve as a reliable surrogate for intact preparations that are generally more difficult to perform and yield a lower percentage of successful experimental recordings.

MATERIALS AND METHODS

Reagents The solution for ganglia dissociation consisted of Trypsin-3X and collagenase type Ⅱ (Worthington Biochem Corp) dissolved in Earle's balance salt solution (Sigma, MO). All cell culture media included D-MEM/F-12 (Gibco, NY), fetal bovine serum (FBS, HyClone Utah), Mito™ + serum extender (Collaborative Biochem Prod, MA), and PSN antibiotic mixture (Gibco, NY). Glass cover slips were coated with poly-*D*-lysine (hydrobromide, Sigma, MO) prior to plating with the isolated NSN. Na-GTP and Na-ATP (Sigma, MO) were added to the pipette solution at time of recording.

Animals Neo (3 – 8 d old, weighing 5 – 8 g), Juv (4 weeks old, weighing 70 – 100 g), and adult (200 – 350 g) Sprague-Dawley® rats of either gender were used to prepare isolated NSN (Neo & Juv) and slices of intact nodose ganglia (Juv & adult). All research animals certified for laboratory use were ordered locally from Harlan (Indianapolis) and housed in the university animal facility for at least 3 d prior to use. Late gestation dams were received several days prior to giving the birth in order to ensure the age of Neo pups. All experimental protocols had previously been approved by the Institutional Animal Care and Use Committee of the School of Science, IUPUI.

Isolated nodose sensory neuron[8] Nodose ganglia were excised surgically from Neo or Juv rats and immediately placed into chilled (4 – 8 ℃) nodose complete medium (NCM, see below). The ganglia were then transferred into a trypsin (Neo) or collagenase (Juv) solution and incubated at 37 ℃ for 30 or 50 min, respectively. The enzyme solution was replaced with 1 mL 37 ℃ NCM. The ganglia were gently titrated with an aspiration pipette and plated onto the cover slips coated with poly-*D*-lysine (0.05 g/L aqueous solution). The neurons were cultured in a 3 % CO_2/97 % air, high humidity environment at 37 ℃ for at least two but not more than 4 h prior to patch recording. NCM contained F-12 medium 90 mL, Mito™ + serum extender 100 μL (v/v, 1∶20), FBS 5 mL, and PSN 1 mL. The F-12 medium contained *D*-MEM/F-12 1000 mL, $NaHCO_3$ 1.2 g, HEPES 7.5 mmol/L with pH adjusted to 7.60 (320 Corning pH meter, NY) using a measured quantity of NaOH 1 mol/L. Bovine albumin (Sigma, MO) 5 mg was added to the NCM just prior to culturing the neurons. The final osmolarity of the culturing solution was about 318 mmol/kg (Vapro™, 5520 Vapor Pressure Osmometer, Wescor Inc, Uath). NCM was prepared under sterile conditions as a stock solution, stored at 4 ℃ and used within 7 d.

Nodose ganglia slice[10] Briefly, unrestrained Juv and adult rats were placed in an air tight induction chamber for inhalation of Metofane® (Methoxyflurane, Schering-Plough, NJ). Lack of reflex response to tail pinch verified sufficient depth of anesthesia and the animals were immediately sectioned at the mid axillary region. The tissue was pinned to a dissection tray; bilateral nodose ganglia and vagus nerves were surgically removed with care; the ganglia were sliced without damaging attached vagus. Then nodose slice with attached vagus was incubated with collagenase type Ⅱ (1 g/L) at 37 ℃ for 40 min followed by a trypsin (0.5 g/L) for 15 to 50 min depending upon the animal age and enzyme activity. The enzyme solution was then replaced with chilled recording solution prior to

transfering to the recording chamber.

Vagal stimulation[10] Nodose ganglia slice with attached vagus was transferred to the recording chambes and gently held in place using a tissue harp, which prevented the tissue moving during the recording. A bipolar stimulation electrode was placed along the distal end of the vagus. At least 10 mm of distance between the recording and stimulation electrodes was required to ensure accurate measurement of CV at room temperature. Short duration ($\leqslant$200 ms) monophasic constant current pulses were utilized for nerve stimulation. The stimulation intensity was dependent upon the preparation and was increased to just beyond threshold prior to recording. Frequency following capacity was investigated using repeated pulse stimulation with no change from the initial stimulus magnitude.

Recording solution The extracellular solution was (in mmol/L) NaCl 137.0, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 2.0, glucose 10.0, and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) 10.0. The pH was adjusted to 7.3 using a measured quantity of NaOH 1 mol/L. The pipette solution was (in mmol/L) NaCl 10.0, KCl 50.0, K_2SO_4 50.0, $MgCl_2$ 5.0, and HEPES 10.0. The pH was adjusted to 7.2 using a measured quantity of KOH 1 mol/L. Just prior to recording, 4.0 mmol/L of 1,2-bis (2-aminophenoxy) ethane-*N*, *N*, *N'*, *N'*-tetraacetic acid tetrapotassium salt (Bapta-K, Sigma, MO), $CaCl_2$ 0.25, Na-ATP 2.0, and Na-GTP 2.0 were added to the pipette solution for a final buffered intracellular calcium concentration of 10 nmol/L. The osmolarity of extracellular and intracellular solutions were adjusted to 310 and 290 mmol/kg, respectively, using *D*-manitol (Sigma, MO).

Whole-cell patch recordings[8,10] Patch electrodes (7052, Corning) were pulled (P-87, Sutter, CA) and polished to 1 – 3 MΩ (Narashigi, MF80). A small plug of Ag-AgCl was used as a bath reference electrode. Somatic AP were studied using the whole-cell current clamp technique with an Axoclamp-2B and Axopatch-1D recording amplifier (Axon Instruments, CA). Two stimulus protocols were applied to evoke somatic AP: brief ($\leqslant$2.0 ms) pulses with sufficient magnitude ($\geqslant$400 pA) to elicit an AP from resting membrane potential (RMP) and longer step depolarizations ($\leqslant$200 pA) applied from a holding potential of –80 mV. Measures of AP waveshape (Fig 1) were performed on all pulse protocol experiments. The entire experimental protocol, data acquisition, storage, display, and preliminary waveform analysis were performed using the pCLAMP 8 software package (Axon Instruments, CA) operating on a PC platform. All experiments were performed at room temperature (21 – 23 ℃).

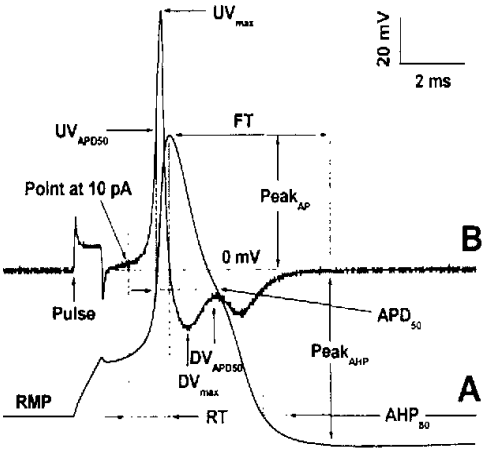

Fig 1. Schematics of AP measurements. All parameters measured in the present experiments were shown in this figure. (A) Action potential recorded from C-type isolated NSN in Neo rat. (B) Displacement current calculated from the same AP (A) using the Axon software packages.

For the confirmation if the distortions of AP recorded using Axopatch-1D occurred during the present experiments, AP from 26 C-type NSN were also studied exactly under the same situation using Axoclamp-2B (Tab 1, C_{-2B}).

Statistics All data were expressed as $x \pm s$ with significance evaluated using *t*-test.

RESULTS

AP from isolated NSN AP from 180 Neo and Juv isolated NSN were recorded. Many of the measured AP parameters from these two age groups were quite similar (Tab 1). 133 NSN exhibited a broad APD_{50}, relatively low UV and a significant hump over the course of repolarization (Fig 2). These neurons were classified as C-type which all exhibited a bimodal trajectory in total outward membrane current when plotted (phase plot) as a function of membrane voltage (Fig 2, inset A). Typically, only one or two AP were recorded from C-type NSN in response to suprathreshold stimulation using the step protocol (Fig 2, inset B). The remaining 47 neurons were classified as A-type and exhibited a narrow

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
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Tab 1. The characteristics of C- (n = 222 in all groups) and A-type (n = 59 in all groups) AP recorded from isolated NSN and nodose ganglia slice by directly stimulating the soma or the attached vagal nerve in Neo, Juv, and adults rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* A-type NSN. $^eP < 0.05$ *vs* Neo-C-type.

Preparation		n	RMP	APD_{50}	$Peak_{AP}$	RT	FT	AHP_{80}	$Peak_{AHP}$	$UV_{APD_{50}}$	$DV_{APD_{50}}$	UV_{max}	DV_{max}	Size
Isolated-Neo	C-	119	−64 ± 5	2.3 ± 0.7^c	50 ± 6^c	1.19 ± 0.17^c	6.7 ± 2.8^c	58 ± 26^c	−65 ± 18	83 ± 48^c	−32 ± 10^c	190 ± 66^c	−57 ± 132^c	36 ± 5
	C_{-2B}	26	−64 ± 5	2.6 ± 0.6	48 ± 4	1.23 ± 0.20	8 ± 4^e	68 ± 21	−65.6 ± 1.9	71 ± 25	−30 ± 10	170 ± 46	−53 ± 13	36.7 ± 2.4
	A-	45	−62 ± 6	0.83 ± 0.15	46 ± 5	0.75 ± 0.12	2.6 ± 0.9	21 ± 10	−67.2 ± 2.1	263 ± 69	−111 ± 24	370 ± 79	−136 ± 53	37 ± 4
Isolated-Juv	C-	14	−63 ± 6	3.8 ± 1.5^c	47 ± 7^b	1.23 ± 0.19^c	13 ± 7^c	80 ± 20^c	−66 ± 4	43 ± 28^c	−29 ± 11^c	150 ± 59^c	−50 ± 17^c	35 ± 5
	A-	2	−63 ± 4	0.83 ± 0.25	43 ± 4	0.72 ± 0.26	2.4 ± 0.8	25 ± 13	−65.9 ± 2.9	258 ± 77	−117 ± 38	372 ± 93	−139 ± 46	38 ± 4
Slice-Juv	C-	21	−64 ± 4	3.7 ± 0.9^c	49 ± 4^c	1.31 ± 0.22^c	8.6 ± 2.9^c	55 ± 28^c	−65 ± 4	77 ± 35^c	−25 ± 9^c	130 ± 40^c	−45 ± 12^c	36 ± 3
	A-	4	−64 ± 6	0.9 ± 0.3	44 ± 6	0.64 ± 0.13	2.3 ± 1.3	24 ± 20	−67.0 ± 2.1	300 ± 86	−127 ± 67	362 ± 118	−158 ± 71	36 ± 3
Vagal Stimul	C-	10	−63 ± 3	3.6 ± 1.1	45 ± 8	2.1 ± 0.5	7.8 ± 2.8	47 ± 21	−66 ± 3	66 ± 29	−30 ± 10	102 ± 32	−46 ± 13	36.7 ± 2.6
	A-	1	−65	0.67	41	0.60	1.03	20.3	−71.4	274	−224	453	−249	36.4
Slice-Adult	C-	42	−66 ± 6	3.3 ± 1.7^c	50 ± 6^c	1.2 ± 0.3^c	8 ± 6^c	64 ± 28^c	−68 ± 3	87 ± 57^c	−31 ± 14^c	162 ± 77^c	−49 ± 28^c	39 ± 4
	A-	8	−63 ± 7	0.84 ± 0.22	43 ± 6	0.69 ± 0.13	1.8 ± 0.6	11 ± 8	−72 ± 4	212 ± 64	−140 ± 52	307 ± 80	−169 ± 54	38 ± 4
Vagal Stimul	C-	26	−66 ± 7	3.2 ± 1.2^c	49 ± 6^b	1.9 ± 0.6^c	8 ± 6^c	45 ± 24^c	−67 ± 3	81 ± 38^c	−30 ± 14^c	142 ± 48^c	−47 ± 14^c	40 ± 3
	A-	3	−61 ± 7	0.84 ± 0.12	42 ± 5	0.81 ± 0.11	1.8 ± 0.6	5.5 ± 1.5	−69 ± 4	261 ± 60	−122 ± 34	319 ± 49	−137 ± 28	39.0 ± 2.6

RMP: Resting membrane potential (mV). APD_{50}: Action potential duration at the point of 50 % height of AP (ms). $Peak_{AP}$: Peak of AP (mV). RT: Rising time of AP (ms). FT: Falling time of AP (ms). AHP_{80}: The time of 80 % recovery of after hyperpolarization (ms). $Peak_{AHP}$: Peak of after hyperpolarization (mV). $UV_{APD_{50}}$: Upstroke velocity at the point of APD_{50} ($V \cdot s^{-1}$). $DV_{APD_{50}}$: Downstroke velocity at the point of APD_{50} ($V \cdot s^{-1}$). UV_{max}: Maximal upstroke velocity ($V \cdot s^{-1}$). DV_{max}: Maximal downstroke velocity ($V \cdot s^{-1}$). Size: μm.

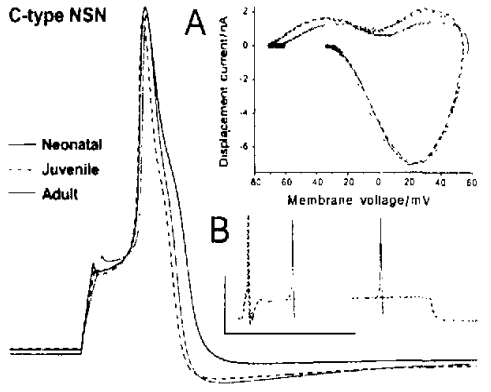

Fig 2. AP were recorded from C-type isolated NSN of Neo, Juv, and adult rats, respectively, by a short pulse applied to the soma. Inset A showed the displacement current phase plots from the each AP, down and upward phases represented the total in and outward currents, and also the depolarizing and repolarizing phase during AP waveform. Inset B displayed the AP recorded from the same NSN by the step protocol (depolarizing currents). The ordinate and abscissa represent the 20 mV and 10 ms, 60 mV and 320 ms for the inset.

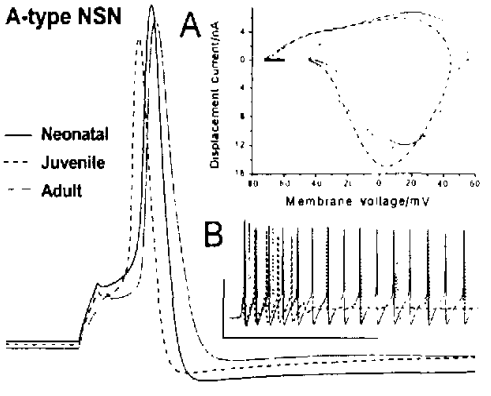

Fig 3. AP were recorded from A-type isolated (Neo and Juv rats) NSN and slices of adult rats, respectively, by a short pulse applied to the soma. Inset A showed the displacement current phase plots from the each AP, down and upward phases represented the total in and outward currents, and also the depolarizing and repolarizing phase during AP waveform. Inset B displayed the AP recorded from the same NSN by the step protocol (depolarizing currents). The ordinate and abscissa represent the 20 mV and 5 ms, 55 mV and 155 ms for the inset.

APD_{50}, greater UV, and lack of a hump over the course of membrane repolarization (Fig 3, inset A). Step depolarizing currents most often elicited repetitive discharge (Fig 3, inset B).

The pooled measurements of RMP, AP peak potential ($Peak_{AP}$), the peak of the after-hyperpolarization ($Peak_{AHP}$), and somatic diameter across populations of C- and A-type isolated NSN from both Neo and Juv rats were not significantly different. The only exceptions were the action potential duration at one-half of the total membrane deflection (APD_{50}), the AP rise time (RT), the AP fall time (FT), the time of 80 % recovery of after-hyperpolarization (AHP_{80}), UV, and DV. Selected AP parameters from isolated NSN such as APD_{50}, RT, $UV_{APD_{50}}$, and $DV_{APD_{50}}$ formed a tight statistical grouping with significant difference ($P < 0.01$) between populations of C- and A-type cells (Tab 1).

AP from nodose slice by soma stimulation A total of 75 AP evoked by somatic stimulation were recorded from Juv ($n = 25$) and adult ($n = 50$) NSN from ganglia slices (Fig 3). Interestingly, nearly all of measured AP characteristics as shown in Tab 1 were quantitatively similar to those recorded from isolated NSN among all populations.

CV and AP from slices by vagal stimulation Forty out of the 75 cells studied in the nodose slices had an intact vagal axon where CV could be recorded. The results indicated that AP with a hump had a broad APD_{50} (> 2.0 ms) with slow $UV_{APD_{50}}$ ($< 100\ V \cdot s^{-1}$) and $DV_{APD_{50}}$ ($< 50\ V \cdot s^{-1}$) (Fig 4, inset A). The average CV for these cells from both Juv and adult nodose slices were less than $1.0\ m \cdot s^{-1}$. The CV in Juv rats [$(0.56 \pm 0.15)\ m \cdot s^{-1}$] was slower than that in adult rats [$(0.9 \pm 0.6)\ m \cdot s^{-1}$, $P < 0.01$]. Those cells without a hump (Fig 5, inset A) had a relatively brief APD_{50} (< 1.0 ms) with greater $UV_{APD_{50}}$ ($> 200\ V \cdot s^{-1}$) and $DV_{APD_{50}}$ ($> 100\ V \cdot s^{-1}$). The A-type CV from Juv and adult rats were all greater than $10\ m \cdot s^{-1}$.

The relationship between CV and frequency response (Fig 4 and 5, inset B) was observed in the present study. The average CV were $(0.74 \pm 0.19)\ m \cdot s^{-1}$ ($n = 11$) with C-type and $(13.1 \pm 2.1)\ m \cdot s^{-1}$ ($n = 3$) with A-type NSN, while the frequency response was (18 ± 11) Hz in C-type and (128 ± 20) Hz in A-type. The CV were very well correlated ($r = 0.966$, $n = 14$, $P < 0.01$) with the frequency response characteristics of nerve evoked AP.

Even though the AP waveform elicited by both somatic and vagal stimulation matched very well either C-

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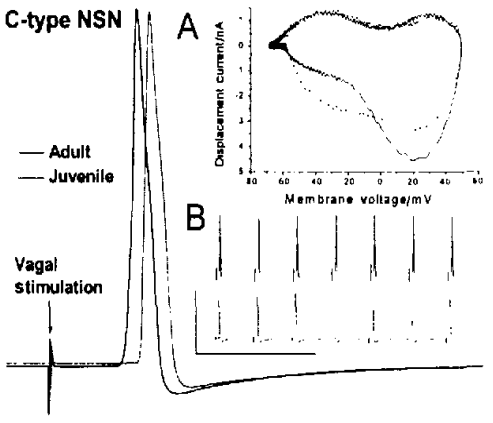

Fig 4. C-type AP was recorded from Juv and adult nodose slice preparation by stimulating the attached vagus. Inset A showed the displacement current phase plot from each AP. Inset B displayed the AP recorded from the same neuron by repeatedly stimulating the vagus. The ordinate and abscissa represent 20 mV and 20 ms, 140 mV and 580 ms for the inset.

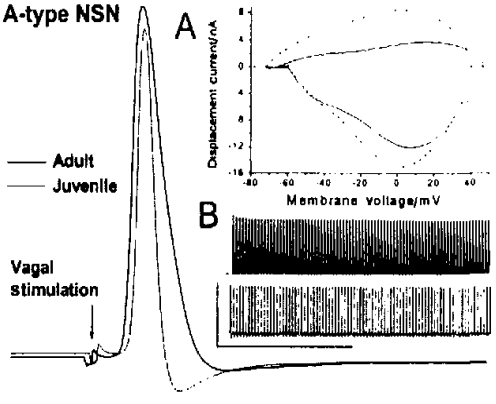

Fig 5. A-type AP was recorded from Juv and adult nodose slice preparation by stimulating the attached vagus. Inset A showed the displacement current phase plot from each AP. Inset B displayed the AP recorded from the same neuron by repeatedly stimulating the vagus. The ordinate and abscissa represent 20 mV and 4 ms, 110 mV and 460 ms for the inset.

or A-type NSN (Fig 6) with nodose slice, the differences were observed in AP firing threshold and the peak of total inward and outward currents as indicated in phase plots (Fig 6 insets). The results showed that the AP firing threshold was more negative (near RMP) when it was elicited by vagal stimulation than that by soma stimulation (over -35 or -50 mV in C- or A-type). The peaks of both inward and outward currents were much lower in C-type compared with those in A-type NSN. Also the peak of inward current appeared at more positive membrane voltage ≥ 20 mV in C-type than A-type near zero mV. While, no matter which age groups, the repolarization hump of C-type was almost located at near zero membrane voltage.

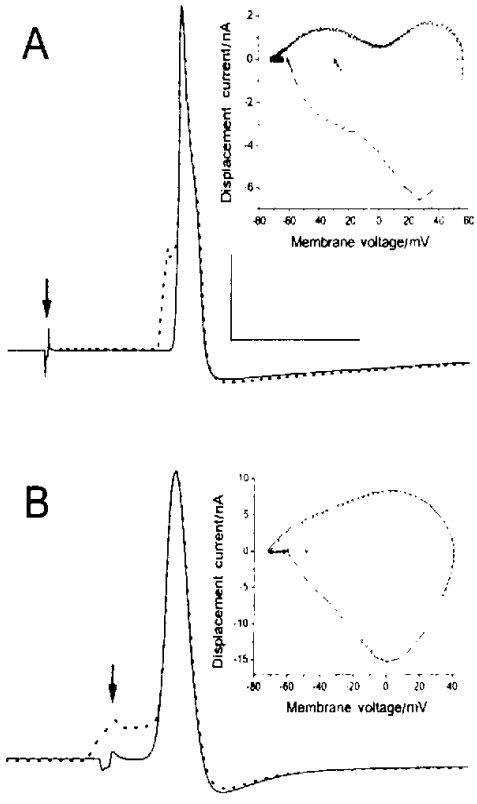

Fig 6. C- and A-type AP were elicited by somatic (dot line) or vagal stimulaion (straight line) in nodose slice. (A) Superimposition of C-type AP by somatic and vagal stimulation with phase plots (inset). (B) Superimposition of A-type AP by somatic and vagal stimulation with phase plots (inset). Arrows show the vagal stimulation. Bars represent 30 mV and 40 ms applied for A, and 30 mV and 2.8 ms for B.

DISCUSSION

Isolated cell and nodose slice preparations were applied in this study. With isolated cell preparation, the procedures of method are much more easier and the cell surface is clean enough for getting a tight seal. It is suitable for both current- and voltage-clamp recordings, but, at most case, only younger animals can be used and afferent CV measurement is not available. On the contrary, if the enzyme incubation time is just right and

proper cell surface cleaning techniques are applied, the giga seal also can be achieved with NSN of adult animals[10]. It is the most important that afferent fiber CV can be recorded on the slice preparation and this is the only reliable identification for sensory neuron classification. In addition, the disease animal model and some research are required for the adult animals. The slice preparation also can be used for all patch configurations, but a little difficulty (lose space clamp) could occurred with voltage-clamp, especially fast current (Na^+) recording because the cell has an axon attached, and connected with each other with sticky tissue around after enzyme. That is the only limitation of slice preparation.

Measure of CV has long been a reliable method for classifying afferent fiber types. Those neurons with AP waveshape characteristics typical of C-type neurons all had CV of less than 1.0 $m \cdot s^{-1}$ and were considered to be unmyelinated afferents. Those neurons with AP waveshape characteristics typical of A-type neurons all had CV of greater than 10 $m \cdot s^{-1}$ and were considered to be myelinated afferents. It has been known that about 90 % of sensory afferents through nodose ganglia are the unmyelinated C-fibers with slow conduction, and the rest of 10 % are the myelinated A-fibers with faster conduction. The ratio of C-fiber versus A-fiber was about 8 - 10/1 and that was the reason why the number of A-type NSN was less frequently observed than C-type. Even if the number of A-type cells was much lower, in the present study, the ratio was still much higher than 10/1 from slices (5/1, 63/12 cells) and isolated cells (3/1, 159/47 cells). With isolated cell or slice recordings, we definitely agreed with that ratio. But the authors were well experienced with the morphological appearances of the cells, so, at certain points, the A-type NSN could be distinguished from C-type and more A-type NSN were collected for the statistics.

In the present study, somatic AP were recorded from identified NSN by afferent fiber CV on intact nodose ganglia of adult rats and these results were consistent with those from isolated NSN[6-8,10], suggesting that the NSN and vagus were well preserved during the preparation. In addition, the AP from Neo were very much resembled with those from adult animals, indicating that the sensory nerve system in Neo rats were well developed before the birth. Over the past decades, there have been numerous research investigations of the electrophysiological and pharmacological properties of NSN[6-8,11]. The development of computational tools for investigating ion channel dynamics and neural functions in NSN has also made possible the integration of cellular data from a range of animal preparations and ages[8]. Cellular electrophysiologists often utilize isolated neurons to perform higher resolution recordings under carefully controlled conditions. Unfortunately, neurons in isolation have no axons and therefore can no longer be classified according to afferent fiber type. To date, it has been unresolved as to whether somatic AP from isolated NSN could adequately represent the natural characteristics of AP discharge in the intact nodose ganglia. Our study was performed in order to make a direct comparison of soma discharge characteristics from both isolated NSN and those within intact adult rat nodose ganglia. With this experiment, two general populations of NSN based upon a bimodal distribution of AP waveshape characteristics were observed and identified mainly by the hump over the course of repolarization in isolated NSN and afferent fiber CV in slices. The results suggested that there were almost no differences among C-types or A-types with all age groups. But the statistical differences were observed between C- and A-type NSN in all AP parameters with the only exception of RMP, $Peak_{AHP}$, and cell size. Compared with A-type NSN, the characteristics of C-type AP could be clearly described according to afferent CV, AP parameters, and phase plot analysis (Tab 2).

Tab 2. Differences of C- and A-type NSN according to the data from CV AP parameters and phase plot analysis. Values present here are average data.

Parameters	C-type AP	A-type AP
CV/$m \cdot s^{-1}$	< 1.0	> 10
APD_{50}/ms	> 2.0	< 1.0
Hump	+	-
$UV_{APD_{50}}$/$V \cdot s^{-1}$	< 100	> 200
$DV_{APD_{50}}$/$V \cdot s^{-1}$	< 50	> 100
FT/mV	< - 35	> - 40
FR/Hz	< 20	> 100
Total IC/nA	< 8	> 10
Total OC/nA	< 2	> 3

FT: firing threshold of AP; FR: frequency response to the continuously frequency vagal stimulation; IC: inward current; OC: outward current.

The previous study[8] showed that Na^+ channel was

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jun; 23 (6)
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mainly involved in AP depolarization, TTX-s/TTX-r Na^+ channels were identified on C-type NSN, while, and only TTX-s Na^+ channel existed in A-type. In addition, multiple channel components were related to AP repolarization, the fast transient K^+ channel component was much less in C-type[12], suggesting that C-type AP should be much more slower in both depolarization and repolarization with broader APD, and less excitable than A-type. Except for CV, The hump over the course of repolarization was the other important identification and constantly observed in all C-type but A-type. Our recently data[13] demonstrated that TTX-r Na^+ channels played more important role than Ca^{2+} channels did to form the hump. While, there was no hump detected on A-type, so, it is still an open question if there were no Ca^{2+} channels expressed on A-type NSN.

The pooled measures of AP waveshape from isolated NSN were correlated with the similar measures of somatic AP waveshape from slices where fiber type was also identified by CV measurements. Therefore, AP data from isolated cellular preparations can serve as a surrogate for recording from adult neurons in intact ganglia. Interestingly, across all age groups and methods of preparation measures of APD_{50}, RT, FT, $UV_{APD_{50}}$, and $DV_{APD_{50}}$ formed a bimodal distribution, which made possible the classification of any neuron as either C- or A-type. Therefore, the quantification of these waveshape parameters in addition to a phase analysis of the total repolarization current can be used to reliably classify isolated NSN. In dorsal root ganglion, cell diameter can be used to differentiate C-type from A-type neurons[14]. However, there was no such relationship in nodose ganglia.

Up to now, we are the very first to present the AP data from identified adult NSN by afferent fiber CV. It is the most important that this enables us to further study the AP and/or ion channel recordings on the same identified adult NSN. That is the reason why Axopatch-1D was selected in the present and future experiments, with which both current- and voltage-clamp configuration can be applied. The AP distortion[15] might occurr with Axopatch-1D. But the present results showed that all AP parameters except for the falling time of AP recorded using Axoclamp-2B and Axopatch-1D had no significant differences probably due to small resistance electrodes used and better compensation in the experiments.

In conclusion, the present study confirmed that NSN could be classified into two general populations according to afferent fiber CV, somatic AP properties, and repolarization hump as recorded from isolated cells and nodose slices. C- and A-type AP from isolated NSN exhibited electrophysiological characteristics that closely followed those recorded in nodose slices. Among the AP parameters, APD_{50}, RT, FT, $UV_{APD_{50}}$, and $DV_{APD_{50}}$ were strongly correlated with CV and robust measures for classifying isolated NSN as either A- or C-type when combined with a measure of the AP repolarization hump, these provide reliable classification of C- and A-type NSN. Therefore, isolated NSN can effectively serve as an experimental surrogate for electrophysiological studies of NSN requiring prior identification of afferent fiber type. Finally, the diameter of NSN could not be used as a reliable indicator of cell types. Importantly, the nodose slice enables us to study the AP and/or ion channels activities on the same identified NSN of adult animals, and it will become more promising and challenging method with the combination use of fluorescent dye labeling technique in order that the electrophysiological studies could be conducted on specific baroreceptor neuron within nodose.

REFERENCES

1 Broussard DL, Altschuler SM. Brainstem viscerotopic organization of afferents and efferents involved in the control of swallowing. Am J Med 2000; 108 (Suppl 4a): 79S – 86S.
2 Berthoud HR, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. Auton Neurosci 2000; 85: 1 – 17.
3 Cervero F. Sensory innervation of the viscera: peripheral basis of visceral pain. Physiol Rev 1994; 74: 95 – 138.
4 Kasparov S, Paton JF. Changes in baroreceptor vagal reflex performance in the developing rat. Pflugers Arch 1997; 434: 438 – 44.
5 Fan W, Schild JH, Andresen MC. Graded and dynamic reflex summation of myelinated and unmyelinated rat aortic baroreceptors. Am J Physiol 1999; 277: R748 – 56.
6 Gallego R, Eyzaguirre C. Membrane and action potential characteristics of A and C nodose ganglia cells studied in whole and tissue slices. J Neurophysiol 1978; 41: 1217 – 32.
7 Puizillout J, Gambarelli F. Electrophysiological and morphological properties of type-C vagal neurons in the nodose ganglia of the cat. J Auton Nerv Syst 1998; 29: 49 – 58.
8 Schild JH, Clark JW, Hay M, Mendelowitz D, Andresen MC, Kunze DL. A- and C-type rat nodose sensory neurons: model interpretations of dynamic discharge characteristics. J Neurophysiol 1994; 71: 2338 – 53.
9 Hay M, Kunze DL. Calcium-activated potassium channels in rat nodose ganglia neurons. Neurosci Lett 1994; 167: 179 – 82.

10 Li BY, Schild JH. Patch clamp electrophysiology in the nodose ganglia of the adult rats. J Neurosci Methods 2002; 115: in press.
11 Stansfield C, Wallis D. Properties of visceral primary afferent neurons in the nodose ganglia of the rabbit. J Neurophysiol 1985; 54: 245-60.
12 Tkatch T, Baranauskas G, Surmeier DJ. Kv4.2 mRNA abundance and A-type K^+ current amplitude are linearly related in basal ganglia and basal forebrain neurons. J Neurosci 2000; 20: 579-88.
13 Schild JH, Li BY. The N-type Ca^{2+} current is not responsible for the repolarization "hump" in the action potential of nodose sensory neurons. FASEB J 2001; 15 (5 Pt 2): A1150.
14 Harper AA, Lawson SN. Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurons. J Physiol (Lond) 1985; 359: 31-46.
15 Magistretti J, Mantegazza M, Guatteo E, Wanke E. Action potential recorded with patch-clamp amplifier: are they genuine? Trends Neurosci 1996; 19: 530-4.

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The 12th China and Japan Joint Meeting on Pharmacology (CJP-2002)

2002, October 27-30 Shanghai, China

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Statistics $[Ca^{2+}]_i$ values were expressed as