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CALCIUM-DEPENDENT AFTER-POTENTIALS IN VISCERAL AFFERENT NEURONES OF THE RABBIT

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SUMMARY

1. Intracellular recordings were made from neurones in nodose ganglia excised from rabbits.

2. In C neurones, 1–60 action potentials were followed by an after-hyperpolarization with a peak amplitude of 16 mV and a time constant of decay ranging from 3 to 10 s. In A neurones, the action potentials were followed only by a brief (up to 50 ms) after-hyperpolarization.

3. The after-hyperpolarization was associated with an increase in the membrane conductance to potassium ions; it reversed polarity at the potassium equilibrium potential.

4. The increase in conductance following the action potentials was blocked by removal of calcium ions, or addition of cobalt to the extracellular solution.

5. Intracellular injection of ethyleneglycol-bis(β-aminoethylether)-N,N'-tetraacetic acid (EGTA) abolished the after-hyperpolarization; intracellular injection of calcium mimicked the after-hyperpolarization.

6. It is concluded that calcium entry during the action potential leads to a long-lasting increase in potassium conductance in visceral afferent C neurones.

INTRODUCTION

The vagus nerve carries general visceral afferent information to the central nervous system. Little is known of the way in which this information is initiated within the viscera, or the mechanisms which underlie its onward transmission in the nucleus of the solitary tract. One way to approach this has been to determine the properties of the membrane of the cell bodies of the afferent fibres in the nodose ganglion. In 1978, Gallego & Eyzaguirre measured the electrical properties of mammalian nodose ganglion cells identified as A type or C type by the conduction velocities and the thresholds of their axons. The ratio of A to C type neurone population (approxi-

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mately 20–80 %) was in good agreement with the ratio of myelinated to non-myelinated sensory fibres of the cervical vagus. This observation has been confirmed later (Higashi & Nishi, 1982). Thus, A type neurones have myelinated axons while the C type have non-myelinated axons. The A and C type neurones had similar passive membrane properties except for the larger capacitance of the C type. They differed, however, in that a majority of the C type had action potentials with an inflexion or hump on the repolarizing phase, and they fired only once or twice at the beginning of a depolarizing current pulse; the somatic spikes of the A type did not have such a hump, and action potentials continued to occur throughout the duration of a depolarizing pulse. From these results it was suggested that the membrane characteristics of the sensory ganglion cells were related to the properties of the peripheral nerves to which they give rise; that is, 'fast' axons originate from fast cells and vice versa (Gallego & Eyzaguirre, 1978; also see Belmonte & Gallego, 1983; Gallego, 1983).

Recently, it has been shown that there are regenerative calcium action potentials in addition to sodium action potentials in many C type neurones of the nodose ganglion of rabbit (Higashi, Shinnick-Gallagher & Gallagher, 1982; Ito, 1982), or in the new-born rat nodose neurones in culture (Baccaglini & Cooper, 1982). Moreover, in rabbit C type neurones the action potentials are followed by a long-lasting after-hyperpolarization (duration several hundred milliseconds) which might be due to an increase in calcium-activated potassium conductance (Higashi *et al.* 1982). This observation may be compatible with the findings from *in vivo* or *in vitro* preparations of cat and rabbit nodose ganglia that repetitive action potentials cause a persistent hyperpolarization lasting for a few to several seconds (Jaffe & Sampson, 1976).

The presence of a calcium-dependent potassium conductance responsible for a long after-hyperpolarization following one or several action potentials has been demonstrated in several nerve tissues (Meech, 1978), but less well characterized in primary sensory neurones (Higashi *et al.* 1982; Ito, 1982; Gallego, 1983). Thus the experiments were performed to describe more fully than previously the mechanism of generation of the after-hyperpolarization which follows the action potentials in C type neurones of rabbit nodose ganglia.

METHODS

Intracellular recordings were made from neurones in isolated superfused nodose ganglia removed from New Zealand White rabbits previously killed by air embolism. The superfusion solution was heated to 37 °C and gassed with 95 % O_2 and 5 % CO_2. It contained (mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $NaHCO_3$, 2·5 and glucose, 11. Intracellular recordings were made with glass micro-electrodes filled with KCl, K citrate or K acetate, having d.c. resistances 20–50 MΩ. In some experiments, calcium ions or ethyleneglycol-bis(β-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA) were injected intracellularly. This was done using double-barrelled intracellular electrodes where one barrel contained KCl (3 M), and the other $CaCl_2$ (10–100 mM) or potassium EGTA (100–300 mM-EGTA in 0·3 M-KOH, pH adjusted to 7·7). Calcium ions were ejected by cathodal current using pulses of 0·5–2 nA and 20–300 ms duration. EGTA was ejected by anodal currents of up to 2 nA (100 ms d.c.). Retaining currents were applied routinely (0·5 nA).

The full details of intracellular recording and current-passing techniques were described previously (Morita, North & Tokimasa, 1982). The membrane input resistance was measured by passing small hyperpolarizing pulses, and conductance changes were calculated in nS. When data were pooled from several cells, the fractional conductance change was used. This was defined as $[(R/R')-1]$ where R is the control input resistance and R' is the changed input resistance (Morita

et al. 1982). Drugs and solutions of changed ionic composition were applied by changing from one to another superfusion solution. Drugs used were tetrodotoxin and sodium cyanide (Sigma).

The infranodose vagus nerve was stimulated on platinum electrodes, and all neurones were identified as A or C cells on the basis of their conduction velocity (Gallego & Eyzaguirre, 1978; Higashi & Nishi, 1982).

RESULTS

Differences between A and C cells

A type neurones had processes conducting at about 8 m/s (range 6–12 m/s). The action potential evoked by direct depolarization of the soma membrane was abolished completely by tetrodotoxin (100 nM). The action potential in A neurones was followed

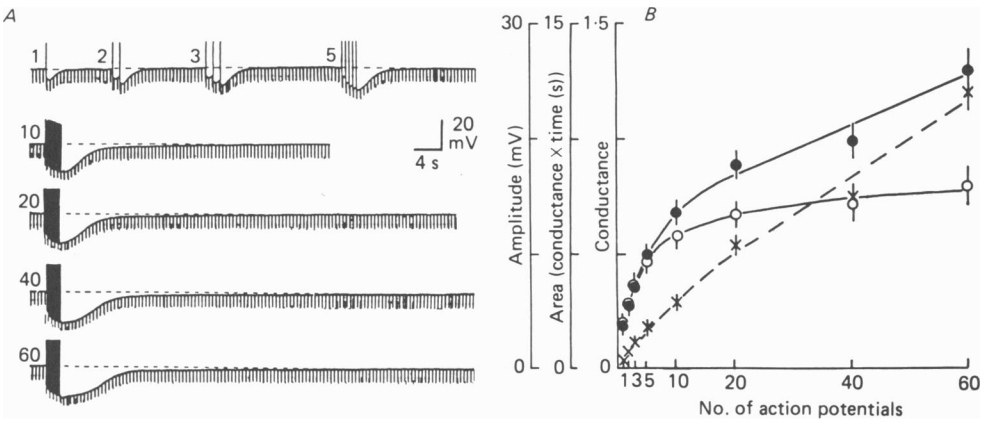

Fig. 1. Relation between number of action potentials and the resultant after-hyperpolarization. *A*, the number of action potentials (1–60) is indicated beside each recording. Note the late component to the decay of the potential change following 10–60 action potentials. *B*, peak amplitude, maximum conductance change and time integral of conductance change as a function of the number of action potentials. The fractional conductance change (●) continued to increase as the number of action potentials increased. The time integral (area) of the conductance change (× — ×) increased almost linearly. The peak amplitude (○) reached a maximum of 16·2 ± 0·8 mV. The vertical bars represent the S.E. of the means of five to sixteen observations.

by a brief (up to 50 ms) after-hyperpolarization, and no prolonged after-hyperpolarization followed a train of action potentials (up to 30 at 10 Hz). C type neurones had action potentials with a shoulder on the falling phase, as originally described by Gallego & Eyzaguirre (1978). An action potential in the soma followed a stimulus to the vagus nerve with a latency appropriate to conduction at 0·4–1·4 m/s. The action potential evoked by direct depolarization of the soma membrane was much reduced in amplitude and rate of rise, but not abolished, by tetrodotoxin (100 nM), as previously described by Higashi *et al.* (1982) and Ito (1982). The residual action potential in the presence of tetrodotoxin was abolished by cobalt (1 mM). All subsequent results described in this paper were obtained during recordings from C neurones in which action potentials were evoked by passing brief depolarizing pulses through the recording electrode.

After-hyperpolarization amplitude

From 1 to 60 action potentials were followed by a prolonged after-hyperpolarization (Fig. 1). This was associated with a fall in input resistance, which still occurred when the membrane potential was prevented from changing by passing appropriate current. The peak amplitude of the after-hyperpolarization increased when the number of spikes used to evoke it was increased, and reached a maximum of about 16 mV when 20 action potentials were used (Fig. 1*A*).

TABLE 1. Relation between peak amplitude of after-hyperpolarization observed and that predicted. The predicted amplitude was calculated from $-30(1-R'/R)$ where R' is the input resistance at peak amplitude and R is the control input resistance. -30 represents the difference in mV between the mean resting potential and mean reversal potential of the after-hyperpolarization. Values are means ± S.E. of means

Number of spikes	After-hyperpolarization peak amplitude		
	Observed	Predicted	*n*
1	4·1 ± 0·7	4·3 ± 0·9	16
2	6·2 ± 0·9	5·9 ± 1·1	15
3	7·6 ± 1·0	7·2 ± 0·9	14
5	9·3 ± 1·1	9·1 ± 1·6	14
10	11·7 ± 1·1	11·3 ± 1·8	13
20	13·1 ± 1·0	13·2 ± 2·5	16
40	14·5 ± 1·1	14·1 ± 3·2	15
60	16·1 ± 1·8	16·4 ± 5·7	7

The maximum conductance change rose steeply as the number of action potentials increased from 1 to 10, and continued to rise less steeply with further increase in the number of spikes (Fig. 1*B*). The time integral of the conductance change between the limits of 0 and 50 s showed an almost linear dependence on the number of action potentials (Fig. 1*B*). The peak amplitude of the after-hyperpolarization was highly predictable from the peak conductance change observed (Table 1), on the assumption that the ions for which the conductance increased had an equilibrium potential 30 mV more negative than the resting potential.

Time course of after-hyperpolarization

The decline of the after-hyperpolarization following a burst of action potentials had two distinct phases (Figs. 1*A* and 2*A*). When a small number of action potentials was used, the decay was a monophasic function of time, but the after-hyperpolarization following 5–60 spikes had an additional very slow component which became more prominent as the number of spikes increased. The conductance increase during the after-hyperpolarization showed a similar biphasic decay. It could be fitted by a single exponential function for less than 20 spikes, but for 20, 40 and 60 spikes a better fit was obtained by the sum of two exponentials (Fig. 2*B*, inset). The time constants ranged from 2·6 s (1 spike) to 4·8 s (10 spikes). Larger after-hyperpolarizations had initial time constants of about 3 s and later components of 6–10 s (Fig. 2).

Effect of membrane potential on after-hyperpolarization

The amplitude of the after-hyperpolarization became progressively less and then reversed when the membrane was hyperpolarized (Fig. 3). The reversal potential was $-91{\cdot}5 \pm 1{\cdot}8$ mV (mean $\pm$ S.E. of mean, $n = 7$). The reversal potential was the same

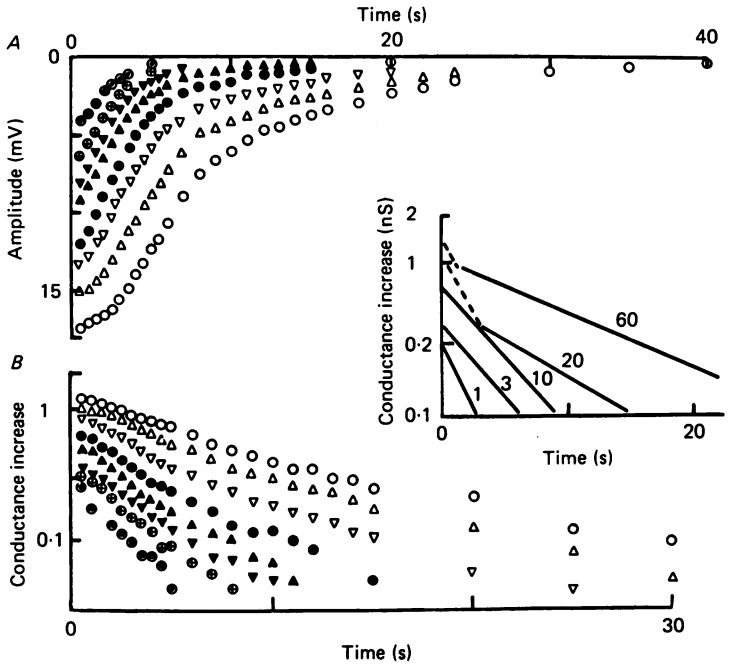

Fig. 2. After-hyperpolarization amplitude and fractional conductance increase as functions of time. *A*, amplitude of after-hyperpolarization at various times after its initiation with 1–60 action potentials. 1 = ⊙, 2 = ⊕, 3 = ▼, 5 (at 2 Hz) = ▲, 10 (at 5 Hz) = ●, 20 (at 10 Hz) = ▽, 40 (at 20 Hz) = △, 60 (at 30 Hz) = ○. Points are means of five to sixteen experiments; S.E. of mean is usually less than the size of the point. *B*, fractional conductance increase calculated from the experiments. Symbols as in *A*. Inset shows best-fit lines to the points shown in 1 *B*, for 1, 3, 10, 20 and 60 action potentials. The conductance change after 20 or 30 action potentials was better fitted by two than one exponential. Membrane potential = $-57{\cdot}3 \pm 2{\cdot}5$ mV, $n = 16$.

whether the after-hyperpolarization followed 1 or 30 spikes. The relation between after-hyperpolarization amplitude and membrane potential was linear in the range of -60 to -100 mV (Fig. 3*B*). At potentials less negative than -50 mV the after-hyperpolarization became smaller.

Effects of changing ion concentrations

These experiments were made using 20 spikes to evoke the after-hyperpolarization.

Potassium. When the solution was changed to one which did not contain potassium ions, the amplitude of the after-hyperpolarization increased by 56 ± 8 % (mean $\pm$ S.E. of mean, $n = 9$). This occurred with no change in the fractional conductance increase,

and with only a small hyperpolarization (3–5 mV) of the resting membrane (Fig. 4). Elevation of the potassium concentration reduced the peak amplitude of the after-hyperpolarization to 61·3±9·7% (10 mM-potassium, $n = 4$) and 35·4±7·3% (20 mM-potassium, $n = 7$). These increases in potassium ion concentration caused

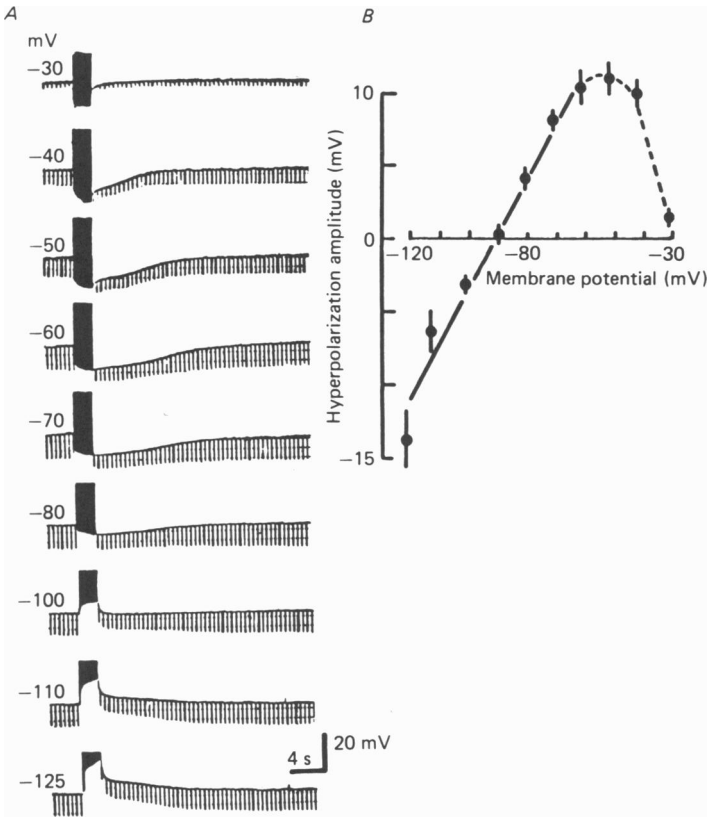

Fig. 3. Amplitude of after-hyperpolarization as a function of membrane potential. *A*, typical records of after-hyperpolarization evoked by 40 action potentials. Note that between −50 and −125 mV input resistance showed little change with membrane potential. *B*, data from six to eight experiments such as those shown in *A* were plotted with their S.E. of means. Mean reversal potential was −92±5·7 mV ($n = 6$).

respective reductions in the resting membrane potential of about 6 and 10mV, and in the input resistance of 30 and 40%. The time constant of decay of the conductance increase was only slightly affected by changing the potassium concentration (control 5·0 s, 10 mM-potassium 4·0 s; control 4·5 s, 20 mM-potassium 3·2 s). The reversal potential of the after-hyperpolarization was reduced when the potassium concentration was increased; a 10-fold change in potassium concentration caused a 51 mV change in reversal potential (Fig. 4*D*).

Chloride. Reduction of the choride concentration to 9 mM (by substituting sodium isethionate for sodium chloride) did not significantly change the reversal potential (−92·7±1·5 mV; mean±S.E. of mean, $n = 3$).

Calcium. When the calcium chloride concentration was doubled (to 5 mM) there was no consistent change in membrane potential, or in the after-hyperpolarization peak amplitude or peak conductance increase. However, the duration of the after-hyperpolarization was increased. In eight experiments the time required for the

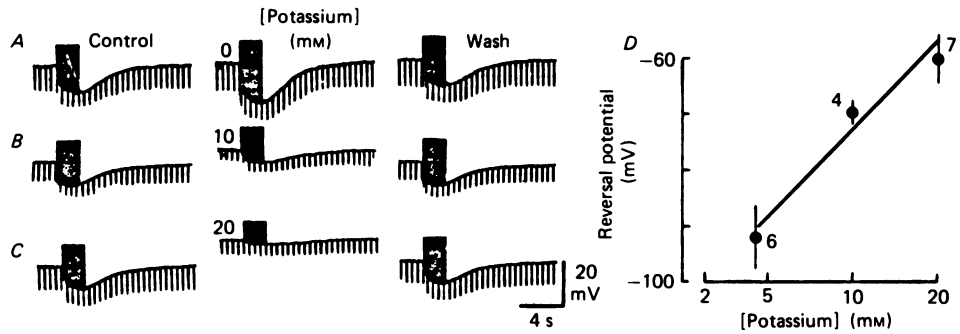

Fig. 4. Effect of extracellular potassium ion concentration on after-hyperpolarization. *A*, peak amplitude became larger in zero potassium solution. Mean increase in amplitude in nine cells was by 56 ± 8·1 %. In the cell shown, resting potential was −61 mV, and this was not changed significantly during 5 min superfusion with potassium-free solution. *B*, 10 mM-potassium depolarized the neurones by 4–6 mV, and decreased peak amplitude to 61·3 ± 9·7 % (n = 4) of control. *C*, 20 mM-potassium depolarized neurones by 6–10 mV, and reduced the peak amplitude to 35·4 ± 7·3 % (n = 7) of control. All measurements were made after 5 min superfusion with the changed potassium solution. *D*, reversal potential of after-hyperpolarization as a function of extracellular potassium concentration. The slope of the line is 51 mV for 10-fold change in K concentration.

after-hyperpolarization to decay to one-half of its peak amplitude was increased to 122 ± 4 % (mean ± S.E. of mean, n = 11) of control in 5 mM-calcium. Reduction in the calcium concentration to 1·2 mM depolarized the resting membrane potential by 0–5 mV in various experiments. This reduced the peak amplitude to 81·9 ± 4·6 %, the peak conductance change to 76·0 ± 6·0 % and the half-decay time to 73·8 ± 10·9 % of their control values (mean ± S.E. of mean, n = 8) (Fig. 5*A*). Complete removal of calcium ions usually depolarized the cell by up to 10 mV. The after-hyperpolarization was abolished in calcium-free solutions.

Effects of cobalt

At concentrations of 10–300 μM, cobalt caused a slight membrane depolarization and greatly reduced or completely abolished the slow after-hyperpolarization. The initial after-hyperpolarization which followed each action potential was unaffected (Fig. 5*A*). This effect was fully reversible. Higher concentrations of cobalt (1–3 mM) depolarized the membrane by 5–10 mV, but completely abolished the slow after-hyperpolarization. The action of such high concentration was only very slowly reversible.

Intracellular injection of calcium

Cells impaled with calcium-containing double-barrelled electrodes appeared to show larger after-hyperpolarizations than cells impaled only with KCl-containing

electrodes. When calcium was injected by pulses exceeding about 1 nA, the membrane was brought to threshold by the injection current. Therefore, in most experiments the after-hyperpolarizations were induced by passing an equal series of depolarizing current pulses through either the potassium chloride-containing barrel or the calcium

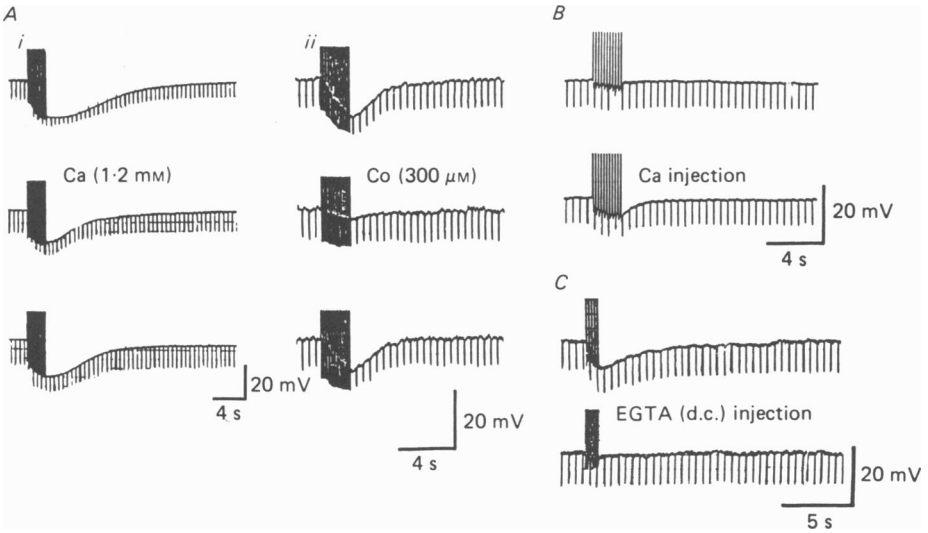

Fig. 5. *A*, effects of low calcium concentration and of cobalt on after-hyperpolarization. In each set of records the top trace is control, the middle during treatment, and the lower trace is recovery. *i*, reducing the calcium concentration from 2·5 to 1·2 mM reduced the amplitude and duration of the after-hyperpolarization. This cell was depolarized by 5 mV in 1·2 mM-calcium (from −61 to −56 mV). *ii*, cobalt (300 μM) for 5 min markedly reduced the after-hyperpolarization. The cell membrane potential changed from −56 to −54 mV. These effects reversed. *B*, effect of intracellular calcium ion injection on a nodose ganglion cell. The cell was impaled with a double-barrelled micro-electrode containing KCl and $CaCl_2$ in a solution containing zero calcium, 10 mM-magnesium, 3 mM-cobalt and tetrodotoxin (500 nM). Resting potential was −54 mV. Upper trace shows that depolarizing pulses (5 Hz) through KCl barrel were ineffective. Lower trace shows that 10 depolarizing pulses through $CaCl_2$ barrel (5 Hz, 1 nA for 30 ms) evoked a small after-hyperpolarization. *C*, effect of EGTA injection on after-hyperpolarization. In each record the after-hyperpolarization was evoked by 10 action potentials (at 10 Hz). Resting potential −62 mV. Upper trace is control. Lower trace shows that after EGTA injection (by passing one current pulse of 1·5 nA for 10 s) the after-hyperpolarization was almost abolished. Resting potential was −56 mV.

chloride-containing barrel. A train of action potentials evoked by depolarizing current pulses passed through the calcium barrel was followed by a larger after-hyperpolarization than when equal current pulses were passed through the potassium barrel. In other experiments, all action potentials were completely abolished by a solution containing no calcium, 10 mM-magnesium, 2–3 mM-cobalt and tetrodotoxin (50 nM) (Fig. 5*B*). Depolarizing pulses carried by potassium now evoked no after-hyperpolarization; however, equal injection of calcium caused a conductance increase and hyperpolarization which declined with a time constant not dissimilar to that of the spike after-hyperpolarization (Fig. 5*B*). The amplitude of the calcium-induced

hyperpolarization was 5·3 ± 1·2 mV (mean ± S.E. of mean, $n = 10$) for calcium injection by 20–30 ms duration pulses of 1 nA delivered at 5 Hz.

Intracellular injection of EGTA

Neurones impaled with EGTA-containing electrodes showed progressively declining after-hyperpolarizations over a period of several minutes to hours. Injection of EGTA

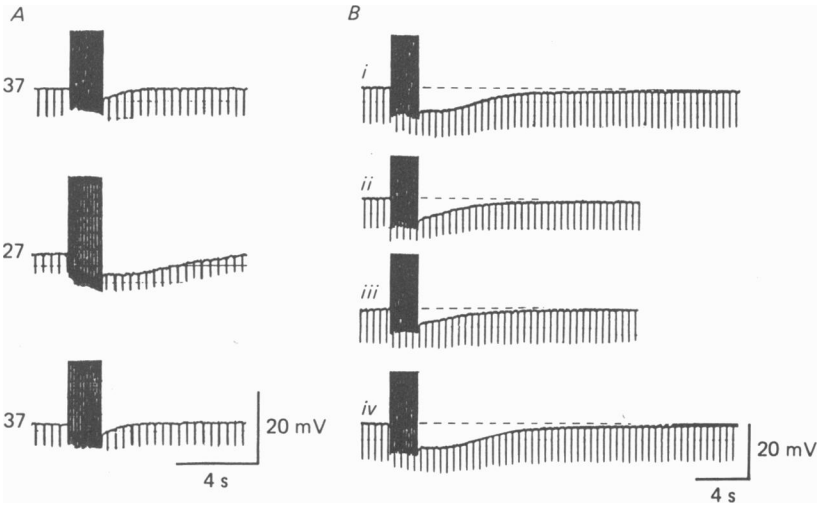

Fig. 6. *A*, effect of rapid cooling on after-hyperpolarization. After-hyperpolarization evoked by 20 action potentials (at 10 Hz). Top trace is control, at 37 °C. Middle trace is after cooling to 27 °C (temperature change occurred in 10 s). The cell hyperpolarized by 3 mV to −59 mV, and the input resistance fell. The after-hyperpolarization was markedly prolonged. Bottom trace is recovery. *B*, sodium cyanide depressed the after-hyperpolarization. After-hyperpolarizations were elicited by 20 action potentials (at 10 Hz). *i*, control. *ii*, 5 min after adding sodium cyanide (10 μM). The cell depolarized by 4 mV (to −59 mV). *iii*, current was passed to restore the membrane potential to −63 mV. Note the marked reduction in the slow component of the after-hyperpolarization. *iv*, recovery after washing out sodium cyanide.

into the cell by a series of hyperpolarizing pulses, or by direct current, reduced the after-hyperpolarization and this effect partially recovered (Fig. 5*C*). When the after-hyperpolarization was reduced following EGTA injection, it could be restored by increasing the number of action potentials.

Effect of cooling

The temperature of the solution adjacent to the nodose ganglion was reduced by 10 °C in approximately 10 s by substituting the inflowing solution by one maintained at a lower temperature. In fifteen of thirty cases this hyperpolarized the neurone (2–16 mV), and this was accompanied by a small fall in resistance (10–20 %). In twelve cells the neurone was depolarized (1–10 mV). It was noticed that cells with a larger after-hyperpolarization were more likely to be hyperpolarized by rapid cooling. The after-hyperpolarization itself was much enhanced and prolonged by the sudden

reduction in temperature (Fig. 6*A*), and this increase progressively disappeared during the next 5–10 min at the lower temperature. The after-hyperpolarization was clearly augmented following even a single action potential, and this slowed the firing rate of the spontaneously firing neurones which were occasionally observed.

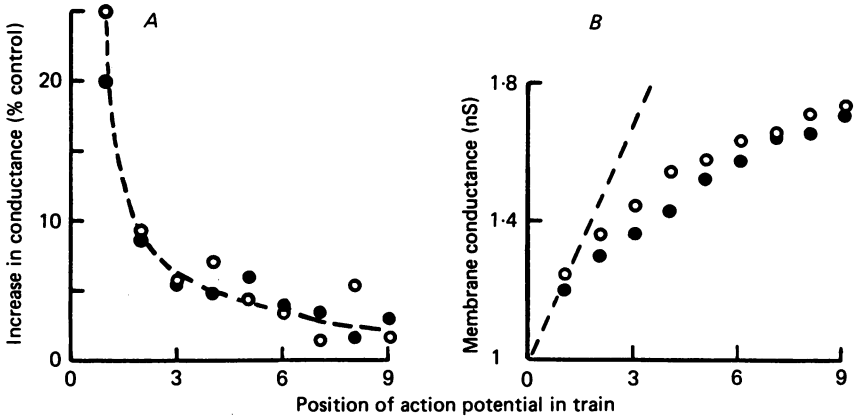

Fig. 7. Time-dependent changes in after-hyperpolarization. *A*, the peak conductance increase following the second, third, fourth... action potentials in a train at 1 (○) and 2 (●) Hz is plotted as a percentage of the conductance increase following the first action potential in the train. Each point is the mean of three to seven cells; S.E. of mean were 1·9–2·4%. Note the marked impairment of the conductance increase mechanism when action potentials were repeated 500 ms or 1 s after each other. *B*, absolute conductance measurements on one cell. Resting potential −58 mV. Input conductance 36 nS. Points show the peak conductance following the first, second, third... action potentials relative to that following the preceding action potential. The dashed line indicates the result expected if each action potential caused the same conductance increase as the preceding one. ○, 1 s between action potentials. ●, 500 ms between action potentials.

Effect of cyanide

When calcium is injected directly into neurones, a major part of its uptake seems to be mitochondrial, and this can be inhibited by metabolic inhibitors such as cyanide (Meech, 1980). We studied the effect of sodium cyanide to ascertain whether such an energy-dependent uptake system might contribute to the decline in the after-hyperpolarization. Sodium cyanide (1–10 μM) never increased but usually decreased the duration of the after-hyperpolarization, without changing its peak amplitude. The effect was reversible after 10–15 min washing, and occurred with no or only a small change in resting membrane potential (2–4 mV) (Fig. 6*B*).

Time-dependent changes in the $g_{K,Ca}$ system

We measured the peak conductance change evoked by the second and subsequent action potentials in a train delivered at 1 or 2 Hz. Fig. 7 shows that the conductance increase evoked by a second action potential was less than that evoked by the control, until the eighth or ninth action potential was almost ineffective. Later action potentials were, of course, evoked when the membrane was already hyperpolarized as a result of the preceding action potentials. However, this degree of hyperpolarization

(from −60 to −75 mV) itself caused little change in the peak conductance change. In Fig. 7*A* the conductance increase caused by the *n*th action potential is shown relative to that induced by the first. It can be seen that it fell steeply as the number of pulses increased. It may be more useful to plot the conductance increase from a given action potential relative to that induced by the immediately preceding action potential, since this should eliminate the cumulative effects of time-dependent restorative processes which follow action potentials much earlier in the train (Fig. 7*B*). Fig. 7*B* shows that at either 1 or 2 Hz, the calcium-dependent potassium conductance became progressively reduced as the number of action potentials increased. This may be due to a reduction in calcium entry as intracellular calcium progressively rose (see review by Hagiwara & Byerly, 1981), or it may represent inactivation of $g_{K,Ca}$.

DISCUSSION

Our results indicate that the cell bodies of nodose ganglion C neurones, though not A neurones, have a prolonged after-hyperpolarization following one or more action potentials. This results from calcium entry during the action potential activating a membrane potassium conductance ($g_{K,Ca}$). The strongest evidence for this assertion is the quantitative agreement between the observed conductance change during the after-hyperpolarization and that predicted on the assumption that the after-hyperpolarization results entirely from an increase in potassium conductance (Table 1), and the symmetrical reversal of the after-hyperpolarization at a potential (Fig. 3) which changed in approximately Nernstein manner with the potassium concentration (Fig. 4). The properties of this $g_{K,Ca}$ appear to be similar to those described in a number of other nerve cells (Meech, 1972, 1978; Latorre & Miller, 1983).

We considered the possibility that the activation of the electrogenic sodium pump may contribute to the generation of the after-hyperpolarizations, as it does in non-myelinated fibres of the rabbit vagus (Rang & Ritchie, 1968) and other tissues (Nakajima & Takahashi, 1966; Baylor & Nicholls, 1969; Kuno, Miyahara & Weakly, 1970). Three observations, however, argue against any significant active pumping. The first is the finding that no after-hyperpolarization remained after removal of calcium ions, or additions of cobalt to the extracellular solution (Fig. 5*A*). The second is the finding that intracellular injection of EGTA abolished the after-hyperpolarization (Fig. 5*B*) whereas intracellular injection of calcium mimicked the after-hyperpolarization (Fig. 5*C*). The third is that there was no depressant effect of cooling on the response (Fig. 6*A*). One might conclude that any activation of electrogenic pumping by 1–60 action potentials is rather small.

The present studies show that when a small number of action potentials was used, the decay of after-hyperpolarization was a monophasic function with time constants of approximately 3 s, whereas larger amounts of calcium entry evoked by 20–60 spikes caused an increase in $g_{K,Ca}$ which decayed biphasically with time constants of about 3 s and 6–10 s (Fig. 2). In *Aplysia* neurones, the time course of decay of $I_{K,Ca}$ or intracellular calcium ($[Ca]_i$) measured by Arsenazo III absorbance, was double exponential (Gorman & Thomas, 1980). In cultured rat muscle cells, the distribution

of the mean open time of calcium-activated potassium channels was also described by the sum of two exponentials, and increasing $[Ca]_i$ increased the number of openings from the longer distribution when compared to the shorter (Barrett, Magleby & Pallotta, 1982). Individual calcium-activated potassium channels typically remain open for only a few to several milliseconds except at very positive membrane potentials (Marty, 1981; Adams, Constanti, Brown & Clark, 1982; Barrett *et al.* 1982). In addition, the activity of the calcium-activated channels was highly sensitive to $[Ca]_i$ (Meech & Thomas, 1977; Gorman & Thomas, 1980; Barrett *et al.* 1982). Thus, one might argue that the prolonged after-hyperpolarization is more likely to reflect the time course of calcium sequestration processes rather than the kinetics of the channels themselves (Meech, 1974; Morita *et al.* 1982; Kuba, Morita & Nohmi, 1983). If this is so, the results of some of the present experiments may be interpreted in terms of calcium movements within the cell.

The findings of the prolonged after-hyperpolarization by rapid cooling do not directly provide a basis to discuss calcium movements within the cell because of possible increases in the amount of calcium entering during the prolonged action potentials at the low temperature. Nevertheless, it has been demonstrated in sympathetic ganglion cells that cooling-induced hyperpolarization was triggered by calcium release from intracellular storage sites (Kuba, 1980). In muscle, rapid cooling is well known to cause the release of calcium from the sarcoplasmic reticulum (Magaribuchi, Ito & Kuriyama, 1973). Thus the transient hyperpolarization and the transient prolongation of the after-hyperpolarization by rapid cooling observed in the present experiments may be comparable to the transient muscle contraction by cooling.

The lack of augmentation of the after-hyperpolarization by cyanide seems to be consistent with a non-mitochondrial calcium sequestration system being responsible for reducing $[Ca]_i$ after it is transiently increased by the action potentials (Brinley, 1978). On the other hand, in *Helix* neurones micro-injected calcium is buffered mainly by mitochondria (Meech, 1980; but also see Akaike, Brown, Dahl, Higashi, Isenberg, Tsuda & Yatani, 1983). If the latter is the case, the inhibitory effect of cyanide may be due to a reduction in $g_{K,Ca}$ as $[Ca]_i$ is excessively increased (Heyer & Lux, 1976*a*,*b*). Unfortunately, this kind of interpretation cannot be taken too far because the source of the calcium ions which activate $g_{K,Ca}$ is not known. Although calcium entry across the plasma membrane may be presumed to be a necessary initiating event, it is possible that this leads to the release of calcium from an internal store and that this released calcium is largely responsible for the long-lasting activation of $g_{K,Ca}$ (Kuba, 1980; Akaike *et al.* 1983; Kuba *et al.* 1983; Nohmi, Kuba & Morita, 1983). In caffeine-treated bull-frog ganglia it seems that the location of the internal store might be the submembrane cisternae which form part of the neuronal endoplasmic reticulum (Fujimoto, Yamamoto, Kuba, Morita & Kato, 1980); caffeine prolonged the after-hyperpolarization in the myenteric plexus (Morita *et al.* 1982) and in nodose ganglion cells (H. Higashi, K. Morita & R. A. North, unpublished observations). In *Helix* neurones, it has been suggested that the site of potential-dependent endogenous calcium release is an intracellular membrane system of endoplasmic reticulum (the submembrane cisternae) and intracellular vesicles, both of which may be fused to the plasma membrane (Akaike *et al.* 1983).

It is also possible that the long time course of the after-hyperpolarization reflects neither the lifetimes of the individual channels nor the period during which free intracellular calcium remains high; a short-lasting rise in intracellular calcium might trigger subsequent metabolic changes which themselves have slow kinetics, and which result in opening of a potassium channel distinct from that which is *directly* sensitive to the intracellular calcium level.

Various sensory modalities are subserved by the vagal C fibres. The present results raise the possibility that the transmission of sensory information may be subject to modulation by means of $g_{K,Ca}$. This is unlikely to occur at the level of the cell soma, which has been studied in the present experiments, but a similar conductance may occur in the membrane of distal parts of the afferent neurone (Gallego, 1983). At the peripheral end this may modulate sensory input by acting to limit the frequency at which the centripetal impulses arise. At the central end the hyperpolarization and conductance increase could contribute to intermittent propagation or reduced transmitter release.

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