A slow voltage-activated potassium current in rat vagal neurons

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SUMMARY

Potassium currents play a key role in controlling the excitability of neurons. In this paper we describe the properties of a novel voltage-activated potassium current in neurons of the rat dorsal motor nucleus of the vagus (DMV). Intracellular recordings were made from DMV neurons in transverse slices of the medulla. Under voltage clamp, depolarization of these neurons from hyperpolarized membrane potentials (more negative than −80 mV) activated two transient outward currents. One had fast kinetics and had properties similar to A-currents. The other current had an activation threshold of around −95 mV (from a holding potential −110 mV) and inactivated with a time constant of about 3 s. It had a reversal potential close to the potassium equilibrium potential. This current was not calcium dependent and was not blocked by 4-aminopyridine (5 mM), catechol (5 mM) or tetraethylammonium (20 mM). It was completely inactivated at the resting membrane potential. This current therefore represents a new type of voltage-activated potassium current. It is suggested that this current might act as a brake to repetitive firing when the neuron is depolarized from membrane potentials negative to the resting potential.

1. INTRODUCTION

Potassium currents play the major role in controlling the excitability of neurons. Different types of potassium current are activated to produce action potential repolarization, to modify transmitter release and to determine the interspike trajectory during repetitive firing (Llinas 1988; Rudy 1988). Some of these currents are activated in response to rises in intracellular calcium while others are activated in response to changes in membrane potential. The voltage-activated potassium currents can be broadly divided into two classes: transient A-type currents and sustained delayed-rectifier type currents (Rudy 1988). These currents are distinguished by their kinetic properties and their sensitivity to tetraethylammonium (TEA) and 4-aminopyridine (4-AP). In general, the transient A-type currents have faster inactivation kinetics and are more sensitive to 4-AP than to TEA, whereas the delayed-rectifier type currents have slower kinetics and are more sensitive to TEA than to 4-AP. It should be noted, however, that there is much diversity in the kinetic and pharmacological properties of potassium currents and classification into one or other category is often difficult (Rudy 1988).

In a study of the electrophysiological properties of neurons of the rat dorsal motor nucleus of the vagus (DMV), we have characterised a transient outward current in these cells which has many of the properties of A-currents in other neurons (P. Sah & E. M. McLachlan, unpublished observation). We noticed, however, that, when this current was blocked by 4-AP, a slow outward current could still be elicited. In this paper we describe the properties of this slow outward current.

2. METHODS

All experiments were done on transverse slices of rat medulla maintained *in vitro*. The methods of obtaining the slices and the recording apparatus have been described previously (Sah & McLachlan, 1991). Rats were deeply anaesthetized with intraperitoneal pentobarbitone (50 mg kg^{-1}) and decapitated. The brainstem was quickly removed and immersed in cold Ringer of the following composition (in millimoles per litre): NaCl, 115; KCl, 5; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4 1.2; $NaHCO_3$, 25, glucose, 10 bubbled with 95% O_2/5% CO_2. Transverse slices (400 μm thick) containing the DMV were then taken with a Vibratome. Slices were allowed to recover for one hour before recording was attempted. Slices were maintained fully submerged in the recording chamber and perfused with Ringer warmed to 28–30 °C. Picrotoxin (100 μM) was routinely added to block the spontaneous inhibitory currents which invariably appeared when KCl filled electrodes were used.

Microelectrodes were fabricated on a Brown–Flaming puller and filled with 0.5 M KCl (resistance 70–140 MΩ). Signals were recorded using a single electrode voltage clamp (Axo Clamp 2a, Axon Instruments), sampled at 0.2 to 5 kHz and stored and analysed on an IBM compatible computer. For voltage-clamp experiments, switching frequencies of 2–3 kHz were used and electrode tips were coated with silicone oil in order to reduce the electrode capacitance. The headstage was continuously monitored to ensure complete settling of the voltage transient between samples. All data (except the current–voltage relation in figure 4*b*) are shown without correction for leakage currents. Leakage currents were generally small as the neurons had a high input

resistance (average input resistance was 293 ± 23 MΩ, $n = 44$). Data are presented as mean ± 1 s.e.m. All chemicals were purchased from Sigma.

3. RESULTS

Figure 1*a* shows the membrane current generated in a rat DMV neuron clamped at −60 mV in response to 300 ms hyperpolarizing voltage steps to membrane potentials of −90 and −120 mV. It can be seen that at the termination of the hyperpolarizing step a transient outward current is activated. Following the step to −90 mV the outward current decays within about 1 s. This current has been characterized in detail elsewhere and shown to have properties similar to those of A-currents (I_A) in several other neurons (P. Sah & E. M. McLachlan, unpublished observation). Following the step to −120 mV, however, a second slower component of the outward current is apparent. In figure 1*b*, this slower component is elicited with a different protocol. The responses to a 50 ms and a 200 ms voltage step to −120 mV have been superimposed. It can be seen that following the brief pulse only the fast transient current is generated, whereas following the longer pulse, a slow component is again apparent. When the cell was stepped to −120 mV for increasing time periods the amplitude of the slow component (measured at 1 s after the peak of I_A) increased exponentially with a time constant of 359 ± 88 ms ($n = 4$).

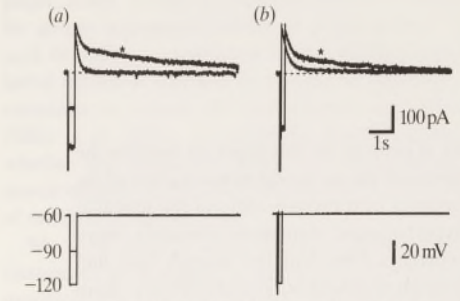

Figure 1. Two transient outward currents in rat DMV neurons. (*a*) Superimposed traces of membrane currents (upper traces) elicited in a cell clamped at −60 mV and stepped to −90 mV and −120 mV for 300 ms (lower traces). The trace obtained by stepping to −120 mV is marked with an asterisk. (*b*) Superimposed traces of membrane currents (upper traces) in a different cell voltage clamped at −60 mV and stepped to −120 mV for 50 ms and 200 ms (lower traces). The trace obtained during the 200 ms step is marked with an asterisk.

The decay of the slow current was well approximated by a single exponential. However, the time constant of decay was very variable ranging between 1.4 s and 5.2 s at −60 mV with a mean value of 3.24 ± 0.23 s ($n = 21$).

The pharmacological profile of the slow current is shown in figure 2. The early outward current, I_A, could be blocked both by 4-AP and by catechol. In contrast, the slow current was not blocked by 4-AP (1–5 mM, $n = 10$; figure 2*a*) and catechol (5 mM, $n = 2$; figure 2*c*); in fact the slow current was larger in the presence of 4-AP. Addition of 4-AP invariably increased the input resistance of the neurons, as can be seen by the smaller inward current during the hyperpolarizing step in figure 2*a*. This increase was variable but was as large as twofold in some cases.

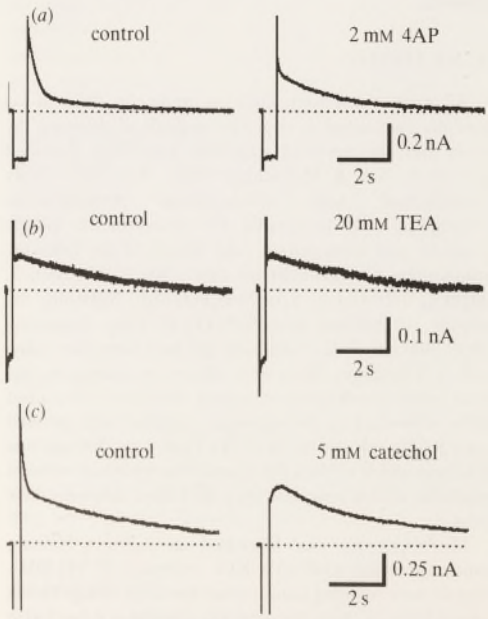

Figure 2. Pharmacology of the slow outward current. In each case the outward current is recorded at −60 mV following a voltage step to −120 mV. (*a*) The effect of 2 mM 4-AP on the transient outward current. Note that I_A is significantly smaller in the presence of 4-AP while the slow outward current is larger. (*b*) The effect of 20 mM TEA. I_A has been blocked by adding 5 mM 4-AP. (*c*) The effect of 5 mM catechol. Note that I_A is completely blocked but the slow current is unaffected.

The slow current was unaffected by TEA (20 mM, $n = 2$; figure 2*b*). In two cells, addition of the calcium channel blocker cobalt (2 mM) abolished the after-hyperpolarization following the action potential but had no effect on the slow current suggesting that it does not require calcium influx for activation. Noradrenaline (100 μM, $n = 3$), serotonin (20 μM, $n = 3$), carbachol (1 μM, $n = 1$) and oxytoxin (1 μM, $n = 2$), were all without effect on the slow current.

The slow current was associated with an increase in membrane conductance (figure 3*a*). The instantaneous current–voltage relation recorded in one cell in 5 mM external potassium is shown in figure 3*b*, *c*. The current rectified at hyperpolarized membrane potentials and had a reversal potential close to the potassium equilibrium potential ($E_K = -88$ mV assuming that intracellular potassium = 140 mM; $n = 4$; figure 3*c*). Changing the extracellular chloride concentration (NaCl in the Ringer being replaced by sodium isethionate) had no effect on the slow current ($n = 3$).

The voltage dependent properties of the slow current are shown in figure 4. Figure 4*a* shows the current generated in one cell following 7 s depolarizing voltage commands from a holding potential of −110 mV. The fast transient current was blocked by 5 mM 4-AP, and

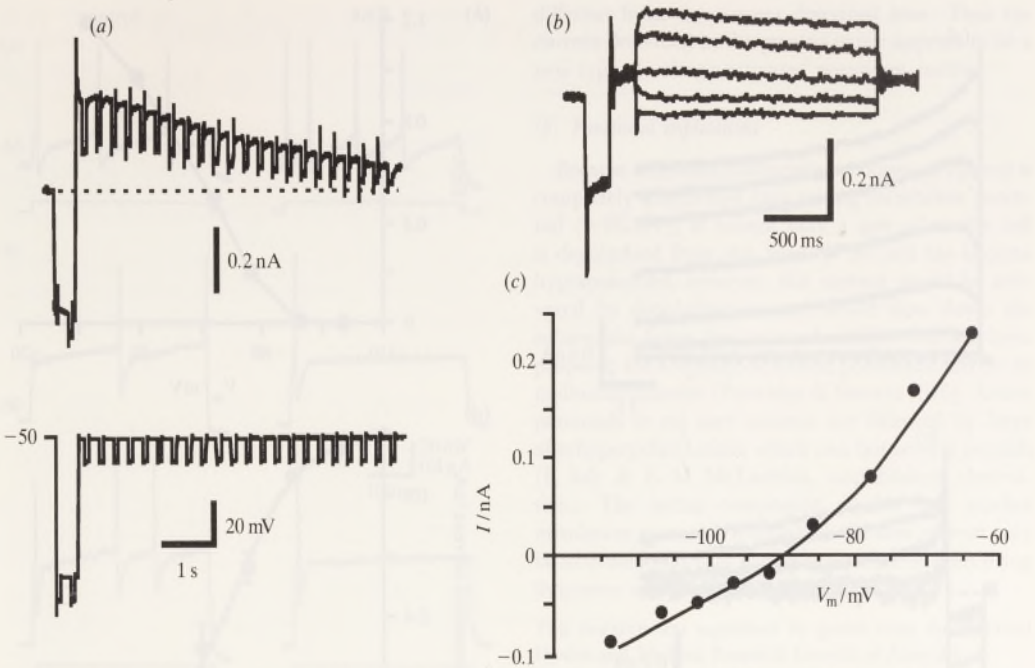

Figure 3. The slow outward current is a potassium current. (*a*) The current generated (upper trace) in response to a step to −125 mV from a holding potential of −50 mV (lower trace). Repetitive 10 mV hyperpolarizing voltage pulses (100 ms) were applied to monitor the membrane conductance. Note that there is an increase in membrane conductance during the slow outward current. (*b*) The current generated in a cell voltage clamped at −76 mV, stepped to −126 mV for 300 ms and then returned to −76 mV for 200 ms to allow any residual I_A to decay, then stepped to −62 mV, −70 mV, −76 mV, −84 mV, and −100 mV. I_A has been blocked with 4-AP (5 mM) and sodium currents with tetrodotoxin (1 μM). (*c*) The instantaneous current voltage relation for the data in (*b*) The solid line has been drawn by eye and shows a reversal potential of −90 mV.

sodium currents by tetrodotoxin (TTX, 1 μM). The peak current–voltage relation (after leak subtraction) for this cell is shown in figure 4*b*. At −80 mV when the current is first activated, the time to peak is of the order of 1 s and this gets faster at more depolarized potentials (figure 4*a*). It should be noted that from this holding potential of −110 mV, the slow current would have been half inactivated (figure 4*d*) and thus 'threshold' for activation may be more negative than measured here.

The voltage dependence of inactivation measured in a different cell is shown in figure 4*c*. The cell was clamped at −50 mV and a 300 ms hyperpolarizing command delivered to different membrane potentials. The normalized inactivation curve averaged for four cells is shown in figure 4*d*. The solid line through the points is the best fit to the equation $I = 1/(1+\exp((V-V')/k))$ where V' is the half inactivation voltage and k is the slope. The half inactivation voltage was −107 mV and the slope was 7.1 mV. In three cells in which the slow current was large and could be measured in the absence of 4-AP, the values for half inactivation and slope were -97 ± 5 mV and 7.3 ± 0.6 mV indicating that 4-AP does not change the voltage dependence of this current.

What might be the functional consequences of this current on the electrophysiological properties of DMV neurons? It is clear that at the resting membrane potential, the current is largely inactivated. Hyperpolarization of the neuron would remove this inactivation. Figure 5 shows the effect of membrane potential on repetitive firing in a DMV neuron at the resting membrane potential (−65 mV, figure 5*a*) and from a hyperpolarized membrane potential (−80 mV, figure 5*b*). Action potentials were elicited by injecting a depolarizing current lasting 400 ms. When the cell was depolarized from −80 mV there was a delay before threshold was reached and the cell fired at a lower frequency. Several action potentials could still be elicited when a larger depolarizing current was injected (figure 5*b*, lower trace). The delayed excitation is due to I_A as has been described in this (P. Sah & E. M. McLachlan, unpublished observation) and other preparations (Cassell *et al.* 1986; Dekin & Getting 1987; Johnson & Getting 1991) and is abolished by 4-AP (figure 5). However, injection of larger depolarizing current in the presence of 4-AP does not elicit any further action potentials. As the slow current is potentiated in the presence of 4-AP (figure 2), it is likely that this curtailment of action potential discharge is due to a larger slow current in the presence of 4-AP. These results suggest that the slow current may act to limit action potential discharge when the neuron is activated from a hyperpolarized membrane potential.

4. DISCUSSION

This paper describes the properties and functional implications of a voltage-dependent potassium current

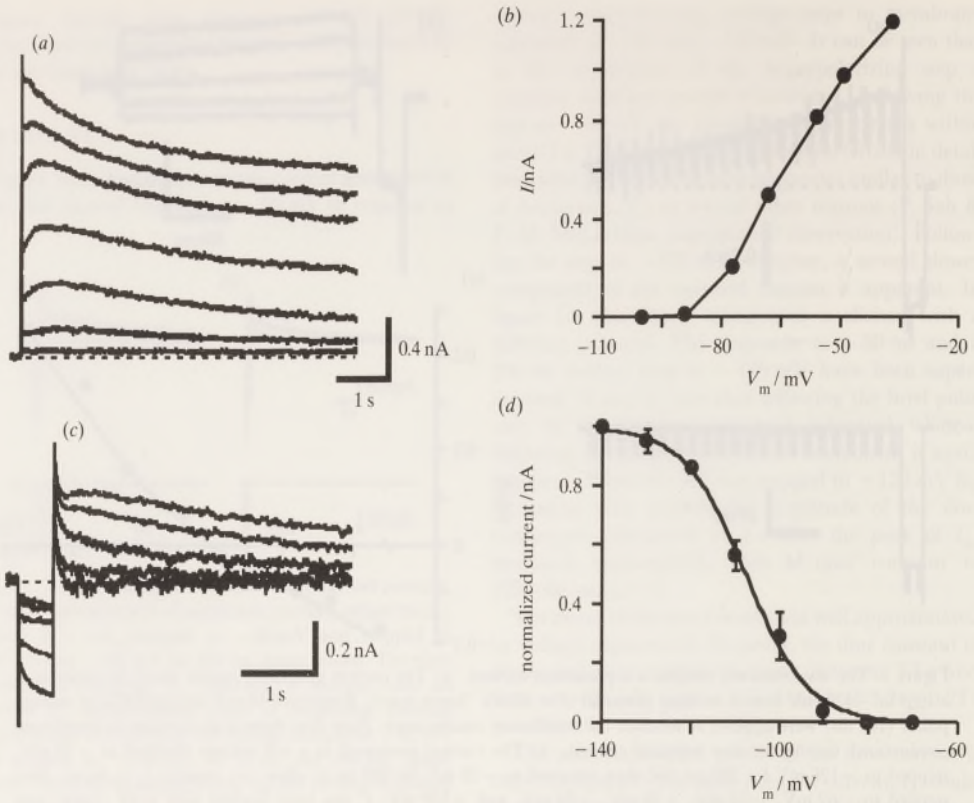

Figure 4. Voltage-dependent properties of the slow potassium current. (*a*) The current generated in a cell clamped at −110 mV and stepped to membrane potentials of −100 mV, −90 mV, −77 mV, −68 mV, −56 mV, −49 mV, and −37 mV. (*b*) The peak current voltage relation for the cell shown in (*b*) after leak subtraction. Leak currents were calculated by appropriately scaling the current generated by a 10 mV depolarizing voltage step. The solid line is drawn by eye. (*c*) Membrane current generated in a cell clamped at −40 mV and stepped to −115 mV, −105 mV, −84 mV, −79 mV, −63 mV and −52 mV. (*d*) Steady-state inactivation curve for average data from four cells. The solid line is the best fit to the equation $I = 1/(1+\exp((V-V')/k))$, where $V' = -107$ mV and $k = 7.1$ mV.

in rat DMV neurons. Depolarization of DMV neurons from hyperpolarized membrane potentials activates two transient potassium currents. One current has a relatively fast activation, decays with a time constant of about 400 ms and is blocked by 4-AP (5 mM) and catechol (5 mM). These properties are consistent with those of A-type potassium currents (Rudy 1988). With larger hyperpolarizations, a second transient outward current becomes apparent. This current was shown to have a reversal potential close to the potassium equilibrium potential indicating that it is predominantly carried by potassium ions. It was insensitive to calcium-channel blocker, cobalt, and could also be recorded in cells loaded with the calcium chelator EGTA (P. Sah, unpublished observations) indicating that it is not calcium dependent. This current activated with a 'threshold' of around −80 mV and inactivated with a time constant of 3.2 s (at −60 mV). The current was half inactivated at −107 mV. At resting potential (−60 mV), the current is fully inactivated.

This current is unlike A-currents because of the lack of blockade by 4-AP (5 mM) and catechol (5 mM). Furthermore, its inactivation kinetics are much slower than those of A-currents recorded in these and other neurons (Rudy 1988). The slow inactivation kinetics of the potassium current and its insensitivity to 4-AP suggests that it is more like the delayed rectifier type current. However, its very slow activation kinetics, its negative activation threshold and the fact that it is not blocked by 20 mM TEA distinguish it from delayed rectifier currents in other neurons (Rudy 1988).

One surprising observation was the increase in amplitude of the slow current in the presence of 4-AP. Because 4-AP increased the input resistance of these neurons, one possibility is that the increase is due to better voltage-clamp control in the presence of 4-AP. However, in view of the fact that the voltage dependence of inactivation with and without 4-AP were not very different this explanation seems unlikely. Similarly the measured reversal potential was very close to the potassium equilibrium potential. Addition of 4-AP also increased the spontaneous synaptic activity in all DMV neurons (P. Sah, unpublished observations). Thus another possibility is that some transmitter is released because 4-AP produces discharge of other neurons in the slice and that substance modulates the slow current. We have shown that noradrenaline, serotonin, carbachol and oxytoxin were

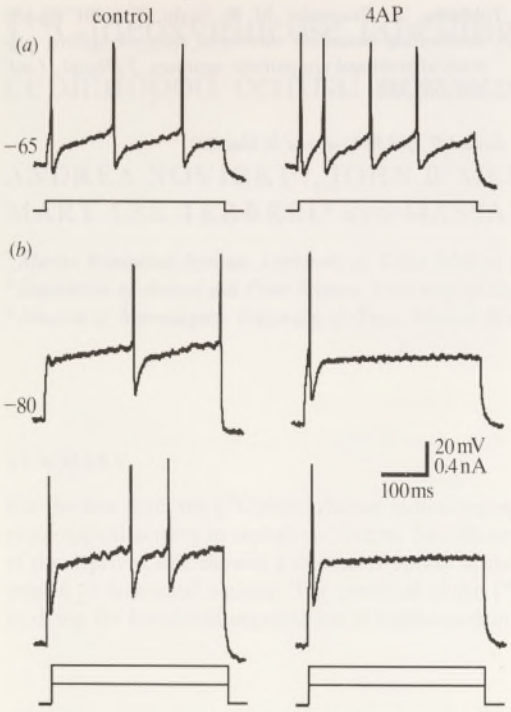

Figure 5. Functional consequences of the two outward currents. (*a*) Membrane potential response in a neuron held at −65 mV and given a 400 ms injection of 0.1 nA (left); when 5 mM 4-AP is added (right) there is a higher frequency action potential discharge due to blockade of I_A. (*b*) Action potentials elicited before (left) and after (right) addition of 4-AP in a cell held at −80 mV and given depolarizing current injections of 0.2 nA (upper traces) and 0.4 nA (lower traces). Note that with a larger current injection, there is an increase in discharge frequency which is abolished in the presence of 4-AP.

all without effect on the slow current and are unlikely to be responsible.

(*a*) *Comparison with other slow potassium currents*

A 4-AP-insensitive slow transient outward current with similar properties has also been described in some guinea pig sympathetic tonic (Cassell *et al.* 1986) and rat hypothalamic (Greene *et al.* 1990) neurons. However, in both these neurons the current had faster inactivation kinetics ($\tau \sim 500$ ms, temperature = 35 °C).

Slow voltage-dependent potassium currents in other neurons have some similarities and differences from the current described here. A similar slow current present in cat parasympathetic neurons (Kumamato & Shinnick-Gallagher 1990) is calcium sensitive. In frog autonomic and sensory neurons (Selyanko *et al.* 1990; Tokimasa *et al.* 1991), the current is sensitive to TEA and only activates at potentials positive to −60 mV. In hippocampal neurons (I_D; Storm 1988), the current is sensitive to low concentrations of 4-AP. The very slow, voltage-dependent potassium current described in uterine (Boyle *et al.* 1987) and atrial (Hume *et al.* 1986) muscle activates over many seconds even at very depolarized membrane potentials and is clearly different from the current described here. Thus the current described in the present paper appears to be a new type of voltage-activated potassium current.

(*b*) *Functional implications*

Because this voltage-dependent potassium current is completely inactivated near resting membrane potential (−60 mV), it cannot play a role when the cell is depolarized from this level. If the cell has become hyperpolarized, however, this current would be activated by depolarization and would slow down the subsequent firing frequency. A similar role has been proposed for a voltage-activated potassium current in molluscan neurons (Partridge & Stevens 1976). Action potentials in rat DMV neurons are followed by large afterhyperpolarizations which can last several seconds (P. Sah & E. M. McLachlan, unpublished observation). The initial component of this AHP reaches membrane potentials from which the slow current may be activated and thus it may contribute to limit firing frequency under certain conditions.

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REFERENCES

Brodwick, M. S. & Junge, D. 1972 Post-stimulus hyperpolarization and slow potassium conductance in *Aplysia* giant neurone. *J. Physiol., Lond.* **223**, 549–570.

Cassell, J. F., Clark, A. L. & McLachlan, E. M. 1986 Characteristics of phasic and tonic sympathetic ganglion cells of the guinea pig. *J. Physiol., London.* **372**, 457–483.

Dekin, M. & Getting, P. A. 1987 In vitro characterization of neurones in the ventral part of the nucleus tractus solitarius. II. Ionic basis for repetitive firing patterns. *J. Neurophysiol.* **58**, 215–229.

Green, R. W., Haas, H. L. & Reiner, P. B. 1990 Two transient outward currents in histamine neurones of the rat hypothalamus *in vitro*. *J. Physiol., Lond.* **420**, 149–163.

Hume, J. R., Giles, W., Robinson, K., Shibitia, E. F., Nathan, R. D., Kanai, K. & Rasmusson, R. 1986 A time and voltage-dependent K^+ current in single cardiac cells from bullfrog atrium. *J. gen. Physiol.* **88**, 777–798.

Johnson, S. M. & Getting, P. A. 1991 Electrophysiological properties of neurons within the nucleus ambiguus of adult guinea pigs. *J. Neurophysiol.* **66**, 744–761.

Kumamoto, E. & Shinnick-Gallagher, P. 1990 Slow inward and late outward currents induced by hyperpolarizing pre-pulses in cat bladder parasympathetic neurones. *Pflügers Arch. Eur. J. Physiol.* **416**, 322–334.

Llinas, R. R. 1988 The intrinsic electrophysiological properties of mammalian neurons: Insights into central nervous function. *Science, Wash.* **242**, 1654–1664.

Partridge, L. D. & Stevens, C. F. 1976 A mechanism for spike frequency adaptation. *J. Physiol., Lond.* **256**, 315–332.

Rudy, B. 1988 Diversity and ubiquity of K channels. *Neuroscience* **25**, 729–749.

Sah, P. & McLachlan, E. M. 1991 Ca^{2+} activated K^+ currents underlying the afterhyperpolarization in guinea pig vagal neurons: A role for Ca^{2+} activated Ca^{2+} release. *Neuron* **7**, 257–264.

Selyanko, A. A., Zidichouski, J. A. & Smith, P. A. 1990 A muscarine-sensitive, slow, transient outward current in frog autonomic neurones. *Brain Res.* **524**, 236–243.

Stansfield, C. E., Marsh, S. J., Halliwell, J. V. & Brown, D. A. 1986 4-Aminopyridine and dendrotoxin induce

repetitive firing in rat visceral sensory neurones by blocking a slowly inactivating outward current. *Neurosci. Lett.* **64**, 299–304.

Storm, J. F. 1988 Temporal integration by a slowly inactivating K^+ current in hippocampal neurons. *Nature, Lond.* **336**, 379–381.

Tokimasa, T., Tsurusaka, M. & Akasu, T. 1991 Slowly inactivating potassium current in cultured bullfrog primary afferent and sympathetic neurones. *J. Physiol., Lond.* **435**, 585–604.

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