Adenosine modulation of potassium currents in postganglionic neurones of cultured avian ciliary ganglia

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1 Potassium currents in cultured postganglionic neurones of avian ciliary ganglia were analysed under whole-cell voltage clamp and their modulation by adenosine determined.

2 In the presence of tetrodotoxin (200 nM), and with moderate holding potentials ($V_h = -40$ mV), the steady-state current-voltage (I/V) curve was N-shaped over the range from -70 mV to $+155$ mV. CsCl (1 M) blocked the current, indicating that it was carried by K^+ . If Ca^{2+} influx was blocked by $CdCl_2$ (500 μ M) then the outward current was reduced and the N-shaped $I-V$ curve lost, indicating the presence of a calcium-activated potassium current ($I_{K(Ca)}$); the remaining current, due to the delayed rectifier (I_K), increased with depolarization up to about a conductance of 10 nS near $+50$ mV. This I_K was 50% activated at about $+20$ mV and 50% inactivated at about -50 mV. Adenosine (10 μ M) had similar effects on the N-shaped I/V curve as did $CdCl_2$, indicating that it blocked $I_{K(Ca)}$. However, adenosine had little effect on the steady-state current in the presence of $CdCl_2$, indicating that it did not much affect I_K .

3 In the presence of tetrodotoxin (200 nM), a large inward current occurred for large hyperpolarizations from a $V_h = -50$ mV. This inward rectifying current (I_{IR}) had a reversal potential near E_K and showed 50% activation at about -130 mV. Adenosine (10 μ M) reduced I_{IR} , by as much as 50% at large hyperpolarizations beyond -80 mV.

4 Relaxations of the outward current on hyperpolarization from $V_h = -30$ mV were blocked by carbachol (10 μ M), had a reversal potential near E_K , and an I/V curve typical of I_M currents. These currents were little affected by adenosine (10 μ M).

5 A fast transient outward current, due to depolarizing pulses from a large $V_h = -110$ mV was observed in the presence of tetrodotoxin (200 nM). This had the characteristics of an I_A current as it could be blocked with 4-aminopyridine (5 mM) and was 50% activated at about -20 mV and 50% inactivated at about -94 mV. The I_A current was reduced by 42% at a depolarization of -20 mV by adenosine (10 μ M).

6 Many neurones possessed a fast transient outward current that was blocked by tetrodotoxin (200 nM). This current could be blocked with 4-aminopyridine (5 mM); it therefore has the characteristics of a sodium-activated potassium current ($I_{K(Na)}$). This $I_{K(Na)}$ was unaffected by adenosine (10 μ M).

7 These results are discussed in relation to the role of adenosine in blocking Ca^{2+} channels and thereby modifying calcium-dependent components of K^+ currents.

Keywords: Synapse; potassium current; ciliary neurones; adenosine; postganglionic neurones; Ca^{2+} channels

Introduction

Endogenous adenosine modulates secretion at synapses (Phillis & Wu, 1981), by acting as an autoinhibitory agent which reduces both the mean quantal content of the synaptic potential and its variance (Ribeiro & Sebastiao, 1987; Bennett *et al.*, 1991). The avian ciliary ganglion provides an opportunity to elucidate the mechanism of action of adenosine as transmission is modulated by endogenous purine nucleotides at this synapse (Bennett & Ho, 1991). Furthermore the synapse possesses giant calyciform nerve terminals that are accessible to single electrode voltage clamp (Martin & Pilar, 1963). Such studies have shown that adenosine hyperpolarizes nerve terminals, suggesting that its action increases the outward potassium currents (Bennett & Ho, 1991). Adenosine hyperpolarizes atrial cells by activating I_M potassium channels via GTP-binding proteins (Kurachi *et al.*, 1986). It also hyperpolarizes both hippocampal and striatal neurones through a Ca -independent I_M type K^+ channel that requires guanosine 5'-triphosphate for activation (Trussell & Jackson, 1987). In addition, adenosine increases a 4-amino pyridine-sensitive potassium current in hippocampal neurones (Gerber *et al.*, 1989). In the present work cultured ciliary neurones have been used to analyse the action of adenosine on the large number of macroscopic K^+ currents which are found in these neurones.

Methods

Tissue culture

White leghorn chicks were used for all experiments. Ciliary ganglia from 7–9-day old chick embryos were dissected free in Ca^{2+} , Mg^{2+} -free Hanks Balanced Salt Solution containing 0.3% bovine serum albumin (BSA), and incubated at $37^\circ C$ for 30 min. No proteolytic enzymes were used. The supernatant was removed and 1 ml of Dulbecco's Modified Eagles' Media (DMEM, Flow, Sydney) supplemented with 2 mM glutamine, 1.00 $u\text{ ml}^{-1}$ penicillin, 50 $\mu\text{g ml}^{-1}$ streptomycin, 10% heat-inactivated horse serum (CSL) and 2% chick embryo extract was added. The ganglia were then mechanically dissociated by repeated trituration through a Pasteur pipette. The resultant single cell suspension was plated onto 13 mm glass coverslips previously coated with poly-L-lysine in 24-well plates (Corning) and maintained at $37^\circ C$ in a 5% CO_2 in air water-saturated atmosphere until required. Neurones were used for whole-cell recording within 12 to 24 h after plating, when they have few processes. These cells were chosen to minimize difficulties involving spatial non-uniformities of membrane potential arising from inadequate space clamp; if these occurred the results were excluded.

Solutions

Electrodes for forming gigaohm seals with the cell membranes of ciliary neurones were fabricated from boro-silicate capillary

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tubing and had resistances of 0.5 to 2.0 M Ω when filled with internal solution (Hamill *et al.*, 1981). For the whole-cell recording presented in this work, the pipette solution consisted of (mM): KCl 115, MgCl₂ 1, HEPES 40, GTP 2, ATP 1 with KOH added to give a pH of 7.3. The bathing solution consisted of (mM): NaCl 140, KCl 5, CaCl₂ 2, MgCl₂ 2, glucose 10, HEPES 10, tetrodotoxin 200 nM and NaOH added to give a pH of 7.3. Variations on these solutions involved addition of KEGTA to the pipette solution, addition of CdCl₂ to the bath solution or omission of tetrodotoxin or CaCl₂ from the bath solution. Such changes are noted in the appropriate legends. All experiments were at room temperature, about 21°C.

Recording

Whole cell patch clamp techniques were used (Fenwick *et al.*, 1982) to voltage-clamp cells. Prior to forming seals the pipette current due to liquid junction potentials within the electrode and bath was nulled and the pipette capacitance compensated. After establishing a seal, the patch membrane was broken under slight negative pressure by a short (0.5 ms) 1.5 V pulse. This negative pressure, which delayed the onset of loss of contact with the cytoplasm, was maintained throughout the course of the experiment. Ion currents were recorded via an Axopatch IC amplifier and a labmaster interface on an IBM-AT using pClamp (Axon Instruments) software. Currents filtered at 2 kHz, were sampled at 0.5 to 6.7 kHz depending on the duration of the voltage clamp command.

Records presented in the figures have had periods of less than 2 ms at the onset and offset of the command pulses removed to eliminate spurious points due to incomplete capacitance compensation. If there were indications of incomplete voltage clamping, due to inadequate space clamp, then the results were rejected. Voltage-clamp control was determined according to several criteria: firstly, smooth voltage-dependent current activation; secondly, lack of excessive delay in onset of current; thirdly, onset and offset kinetics that were dependent on voltage but not on the amplitude of the current. Isopotentiality of the neuronal membrane was determined by testing the exponentiality of the capacitive transient.

Adenosine application

Micropipettes with tip diameters of about 10 μ m were used to deliver the drug solutions. The pipettes were filled with bath solution together with adenosine to a final concentration of 10 μ M to 15 μ M. The tips of the micropipettes were positioned 20 to 50 μ m from the neurone cell body and adenosine pres-

sure ejected at 5 kPa. During application of the adenosine, currents were evoked every 20 s. Neurones were required to show stable current magnitudes for at least 2 min before application of the adenosine, otherwise the results were rejected.

Drugs

The drugs used and obtained from Sigma, were: tetrodotoxin, tetraethylammonium chloride, adenosine and 8-phenyltheophylline.

Statistical analysis

Values are expressed as mean $\pm$ s.e. and *n* indicates the number of neurones.

Results

The delayed rectifier (I_K) and calcium-activated potassium currents ($I_{K(Ca)}$): effects of adenosine

Depolarizing voltage-command steps of 470 ms duration from a holding potential (V_h) of -40 mV gave outward currents of the kind shown in Figure 1a in all cultured ciliary neurones studied ($n = 20$ neurones). The current at the end of the command step increases up to depolarizations of about $+60$ mV; thereafter the current declines to reach a minimum at about $+100$ mV from which it increases again (Figure 1b). This N-shaped current-voltage curve is typical of that for neurones in which a large component of the outward current passes through calcium-activated potassium channels ($I_{K(Ca)}$): as $I_{K(Ca)}$ decreases markedly for depolarizing commands close to the calcium equilibrium potential, a minimum is reached near this potential on the current-voltage relationship (see for example Hermann & Gorman, 1981; Galvan & Sedlmeir, 1984).

The delayed rectifier currents (I_K) were separated from $I_{K(Ca)}$ by blocking the influx of Ca^{2+} ions with 500μ M Cd^{2+} . Figure 2a shows that most of the outward current was blocked by the addition of Cd^{2+} , indicating that most of this current is carried by $I_{K(Ca)}$. The current-voltage relation for the Cd^{2+} -sensitive current and the Cd^{2+} -insensitive current (Figure 2b) show that the Cd^{2+} -sensitive current declines to less than 1 nA about the expected calcium-equilibrium potential (near $+100$ mV) whereas the Cd^{2+} -insensitive current increases continually with depolarization. If the Cd^{2+} -insensitive current is taken as I_K , then the steady-state conductance for the I_K channel increased from -50 mV depolarization to reach a value of about 8.5 nS (± 1.4 nS, $n = 3$) at $+50$ mV (Figure 2c).

Figure 1 (a) Shows a set of voltage-clamp currents for a ciliary neurone during command pulses at 470 ms duration to the different levels indicated by the rectangular traces; the holding potential was -40 mV; (b) shows the current-voltage relation determined from the currents at the end of the traces for the neurone in (a).

Figure 2 Separation of the total delayed outward current in a ciliary neurone into delayed rectifier (I_K) and calcium-activated potassium ($I_{K(Ca)}$) components: (a) shows the voltage-clamp currents for a ciliary neurone during a depolarizing command pulse of 470 ms duration (indicated by the rectangular trace) before (upper trace) and during the application of Cd^{2+} ($500 \mu M$; lower trace) by pressure ejection; holding potential is -40 mV; the remaining (middle) trace gives the difference between the currents before and after Cd^{2+} ; (b) gives the current-voltage relation for the current remaining after addition of Cd^{2+} (○, I_K) as well as the current-voltage relation for the current due to subtracting that remaining after Cd^{2+} from the control current (●; $I_{K(Ca)}$). In (c) the steady-state conductance (G_K) for the I_K in (b) according to $G_K = I_K / (V - V_K)$, where $V_K = -75$ mV is shown; the curve has been drawn according

to $G_K = G_K \left\{ 1 + \exp \frac{Ze}{kT} (V_o - V) \right\}^{-1}$ in which $G_K = 8.6$ nS, $V_o = 0$ mV, $Z = 2.5$, $\frac{Ze}{kT} = \frac{Zf}{RT} = 0.1$.

Figure 3 Activation and inactivation of the delayed rectifier current (I_K): (a) gives a set of voltage-clamp currents for the same ciliary neurone during depolarizing commands indicated by the rectangular traces from a holding potential of -80 mV; (b) shows a set of voltage-clamp currents during a depolarizing command to $+50$ mV from different holding potentials (indicated by the rectangular traces). In (c) the activation curve for the steady-state current (at 940 ms) for this neurone (○; holding potential $V_h = -80$ mV) is shown, derived from the results in (a), as well as an inactivation curve (●; depolarization to $+50$ mV) derived from the results in (b); the graph shows the respective current values (*I*) each normalized to their maximum saturated values (I_{max}). The inactivation curve has been drawn according to $h = (1 + \exp (V - V_h)/V^*)^{-1}$ in which V_h (the potential for *h* of 0.5) is -50 mV and V^* is $+10$ mV. The activation curve has been drawn according to $h = (1 + \exp (V_h - V)/V^*)^{-1}$ in which V_h is $+17$ mV and V^* is $+15$ mV. Cd^{2+} ($200 \mu M$) present throughout to block $I_{K(Ca)}$. There was no Ca^{2+} in the bath solution and KEGTA (10 mM) substituted for KCl (10 mM) in the pipette solution.

Figure 4 Effects of adenosine on the calcium-activated potassium current $I_{K(Ca)}$. In (a) is shown a set of voltage-clamp currents in a ciliary neurone during command pulses of 5 s duration to the different levels indicated by the rectangular traces from a holding potential of -50 mV both (i) before and (ii) after the addition of adenosine ($15 \mu M$) to the bath. In (b) are shown the current-voltage relation determined from the currents at the end of the traces in (a) for control (●) and in the presence of adenosine (○); vertical bars give $\pm$ s.e. mean ($n = 5$ cells).

Figure 5 Effects of adenosine on the delayed rectifier current I_K . In (a) are shown voltage-clamp currents in a ciliary neurone during depolarizing command pulses of 250 ms duration to -20 mV from a holding potential of -110 mV before (i) and during (ii) the addition of adenosine ($10 \mu\text{M}$) by pressure ejection; (b) shows the current-voltage relation determined from the currents at the end of the traces like those for the cell in (a) for control (●) and in the presence of adenosine (○). There was no Ca^{2+} in the bath solution and EGTA (10 mM) substituted for KCl (10 mM) in the pipette solution.

Figure 6 The presence of inward rectifier currents (I_{IR}) in ciliary neurones; (a) a set of voltage-clamp currents in a ciliary neurone during hyperpolarizing command pulses of 470 ms duration to the different levels (mV) indicated by the rectangular traces; the holding potential was -50 mV. The current-voltage relation is shown in (b) determined from the currents at the end of the traces for the neurone in (a). The activation curve for the steady-state current derived from the results in (a) is shown in (c); the graph shows the respective current values (I) each normalized to their maximum saturated values (I_{max}); the curve has been drawn according to $h = (1 + \exp(V - V_h)/V^*)^{-1}$ in which V_h is -127 mV and V^* is 14 mV. CdCl_2 ($200 \mu\text{M}$) but no Ca^{2+} was present in the bath solution; KEGTA (10 mM) substituted for KCl (10 mM) in the pipette solution.

The activation and inactivation of the steady-state Cd^{2+} -insensitive current, I_K was determined for a cell in which large currents were generated (Figure 3a). Successively larger command depolarizations from a holding potential of -80 mV gave successively larger currents up to about $+70$ mV when saturation was reached; 50% activation occurred at about $+20$ mV (± 6 mV, $n = 3$) (Figure 3c). An inactivation curve was also determined for this steady-state current by using a depolarizing command of $+50$ mV from successively more positive holding potentials from -100 mV (see Figure 3b). Figure 3c shows that maximum currents are obtained for holding potentials more negative than -80 mV and that 50% inactivation occurs at about -50 mV (± 8 mV, $n = 3$).

Adenosine ($10 \mu\text{M}$) had similar effects on the steady-state current generated by 5 s depolarizing pulses from moderate-size holding potentials in the 5 neurones studied as did the addition of Cd^{2+} (compare Figure 4a with 2a). Thus the slow increase in outward current in about the first second following depolarization, which is due to $I_{K(\text{Ca})}$, was removed by adenosine (Figure 4b). The current-voltage relation then reverts to that for I_K in the presence of adenosine and is, therefore, similar to that in the presence of Cd^{2+} (compare Figure 4 current-voltage relation with that in Figure 2b). Adenosine had very little effect, if any, on the delayed rectifier, I_K (Figure 5a); this current was increased by, at the most, 40 pA (± 10 pA, $n = 5$) over the voltage range from -60 mV to $+10$ mV (Figure 5b).

Inward rectifier and carbachol-sensitive potassium currents: effects of adenosine

Large hyperpolarizing command voltages from a V_h of -50 mV gave very large inward currents (Figure 6a), indicating the existence of an anomalous inward potassium rectifier (I_{IR}) in all neurones studied. The current-voltage curves for this channel showed a steep rise in the inward current for hyperpolarizations beyond about -90 mV (Figure 6b). Extrapolation of the linear part of this curve intersected the voltage axis at about the potassium reversal potential (Figure 6b). An activation curve for this current showed that 50% activation occurred at -127 mV (± 20 mV, $n = 4$) (Figure 6c). Adenosine decreases the currents through the inward rectifier (Figure 7a) by amounts which increase with hyperpolarization in the 4 neurones examined (Figure 7b), so that at about -100 mV over 50% of the current has been reduced.

Potassium currents that are blocked by the addition of carbachol ($10 \mu\text{M}$) were also present in all ciliary neurones (Figure 8a). These I_M currents can be observed as outward relaxations during hyperpolarizing steps from a holding potential of about -30 mV (Figure 8a). The I_M current, measured as the

Figure 7 Effects of adenosine on the inward rectifier current I_{IR} : (a) shows voltage-clamp currents during hyperpolarizing command pulses of 490 ms duration to -90 mV from a holding potential of -30 mV before (ii) and during (i) the addition of adenosine ($10 \mu\text{M}$) by pressure injection. The current-voltage relation is shown in (b) determined from the currents at the end of the traces like those for the cell in (a) for control (●) and in the presence of adenosine (○). There was no Ca^{2+} in the bath solution and KEGTA (10 mM) substituted for KCl (10 mM) in the pipette solution.

difference between the initial current (I_{in}) and steady-state current (I_{ss}) during the hyperpolarizing steps, is relatively unaffected by adenosine ($n = 4$ neurones; Figure 8b).

Transient I_A and sodium-activated potassium currents: effects of adenosine

In the presence of tetrodotoxin (200 nM), an early transient outward current generated by depolarizing pulses to about -35 mV from large holding potentials of about -110 mV is observed in some cells (8 out of 13 neurones; Figure 9a). This current has the inactivation characteristics of the I_A current (Figure 9b) and is blocked by 4-aminopyridine (Figure 9a). Adenosine ($10 \mu\text{M}$) decreased this current by 42% ($\pm 8\%$, $n = 5$; Figure 9c). Another early transient outward current can also be observed in some cells (7 out of 13 neurones) but only in the absence of tetrodotoxin (Figure 10). This transient was eliminated by 4-aminopyridine. Adenosine had no effect on this sodium activated potassium current ($I_{K(Na)}$) in the 7 neurones in which it was observed.

Discussion

This work shows that cultured postganglionic neurones from nine-day embryonic avian ciliary ganglia possess a large

number of different potassium channel types. The N-shaped current-voltage curve for the quasi steady-state current observed at depolarizations from a moderate holding potential after about 500 ms was due to co-activation of two different potassium currents, as is the case in rat sympathetic neurones (Galvan & Seldmeir, 1984) and molluscan neurones (Hermann & German, 1981). One of these was a delayed rectifier current (I_K), having similar characteristics to that of I_K in sympathetic neurones (Galvan & Seldmeir, 1984), which inactivates over seconds. The other component of the N-shaped current-voltage curve was due to a calcium-activated potassium channel ($I_{K(Ca)}$), as it could be removed by blocking the inward flux of calcium ions with CdCl_2 . This current had similar characteristics to those of $I_{K(Ca)}$ in bullfrog sympathetic ganglia, in that it activated soon after a depolarizing command (Lancaster & Pennefather, 1987). The channels responsible for $I_{K(Ca)}$ have been identified directly in ciliary neurones by patch-clamp methods (Gardner, 1986), as BK^+ or maxi- K^+ channels (Latorre *et al.*, 1989) and have a large conductance (193 pS) as well as an absolute requirement for $[\text{Ca}^{2+}]_i$. Adenosine considerably reduces $I_{K(Ca)}$, probably as a consequence of reducing the inward flux of calcium ions in the same way as adenosine reduces $I_{K(Ca)}$ in dorsal root ganglion cells (Scott & Dolphin, 1987). $I_{K(Ca)}$ contributes significantly to the after-hyperpolarization of the action potential in hippocampal neurones (Wong & Prince, 1981) and to the repolari-

Figure 8 Effects of adenosine on the I_M current: (a) shows voltage-clamp currents during hyperpolarizing command pulses of 490 ms duration to -50 mV from a holding potential of -30 mV before (ii) and during (i) the addition of adenosine ($10 \mu\text{M}$) by pressure injection. In (b) is shown the current-voltage relation determined from the I_M currents measured as the slow relaxation at the beginning of the pulse ($I_{ss} - I_{in}$); (●) control I_M and (○) I_M in the presence of adenosine. There was no Ca^{2+} in the bath solution and EGTA (10 mM) substituted for KCl (10 mM) in the pipette solution.

Figure 9 Effects of adenosine on the I_A current. In (a) are shown voltage-clamp currents during depolarizing command pulses of 350 ms duration to -35 mV from a holding potential of -110 mV before (i) and during (ii) the addition of 4-aminopyridine (5 mM) by pressure ejection. Inactivation and activation curves are shown in (b) for the fast transient current derived from results for the cell in (a); the activation graph (●) shows the respective current values (I) each normalized to their maximum saturated values ($I_{\max}$); the curve has been drawn according to $h = (1 + \exp(Vh - Vh)/V^*)^{-1}$ in which Vh is -94 mV and V^* is $+10$ mV; the activation graph (○) shows the respective current values (I) each normalized to their maximum saturated values ($I_{\max}$); the curve has been drawn according to $h = (1 + \exp(Vh - V)/V^*)^{-1}$ in which Vh is -20 mV and V^* is $+7.5$ mV. In (c) are shown voltage-clamp currents during depolarizing command pulses of 300 ms duration to -20 mV from a holding potential of -120 mV before (i) and after (ii) the addition of adenosine ($10 \mu\text{M}$) by pressure ejection. There was no Ca^{2+} in the bath solution and EGTA (10 mM) substituted for KCl (10 mM) in the pipette solution.

zation of the action potential in sympathetic neurones (Lancaster & Pennefather, 1987). The after-hyperpolarization in hippocampal neurones is greatly reduced by noradrenaline acting to block $I_{K(\text{Ca})}$ (Madison & Nicoll, 1986; Lancaster & Adams, 1986), which adenosine might be expected to do on ciliary neurones.

An anomalous or inward rectifying potassium current (I_{IR}) is present in all the cultured ciliary neurones becoming activated at hyperpolarizing levels near -70 mV, like that of I_{IR} in guinea-pig olfactory cortical neurones (Constanti &

Figure 10 The presence of sodium activated potassium currents $I_{K(\text{Na})}$ in ciliary neurones. Shown are voltage-clamp currents during depolarizing command pulses of 250 ms duration to -20 mV from a holding potential of 110 mV before (c) and during (t) blocking the entry of sodium ions with tetrodotoxin (200 nM) by pressure ejection.

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Galvan, 1983). Another outward current was revealed during hyperpolarizing steps from a depolarized holding potential. This was distinguishable from the anomalous rectifier by its kinetics and less negative activation range; in addition it was blocked by carbachol and can therefore be identified as an I_M current (Adams *et al.*, 1982; Brown *et al.*, 1982). Adenosine reduced I_{IR} by up to 50% at large hyperpolarizations. This may be contrasted to the effects of opioids in some neurones, which is to increase I_{IR} (Loose & Kelly, 1990). Adenosine had little effect on I_M .

Two fast outward transient currents, peaking in less than 5 ms and then decaying with a time constant of about 20 ms, were also observed for depolarizing steps from large holding potentials. These transients could be distinguished from each other as tetrodotoxin blocked one of them completely, indicating that it was a sodium-activated potassium channel ($I_{K(\text{Na})}$; Hartung, 1985; Dryer *et al.*, 1989). The tetrodotoxin-insensitive transient, which was blocked by 4-aminopyridine and had an activation range from -50 mV to 0 mV, had the characteristics of an I_A current, although somewhat faster than the I_A current transients observed in other excitable cells such as hippocampal neurones (Segal *et al.*, 1984; Zbicz & Weight, 1985). In some neurones only the $I_{K(\text{Na})}$ was observed and in others only I_A , whereas a few neurones possessed both $I_{K(\text{Na})}$ and I_A . Adenosine did not affect $I_{K(\text{Na})}$ but it consistently reduced I_A , in much the same way as it does a similar transient potassium current in dorsal root ganglion cells (Scott & Dolphin, 1987), but in contrast to the effects of adenosine in increasing I_A in hippocampal neurones (Gerber *et al.*, 1989). The rate of action potential firing is regulated by I_A and this rate is higher in tonic-firing sympathetic neurones than in phasic-firing sympathetic neurones (Cassell *et al.*, 1986). It will be of interest to see if adenosine derived from endogenously released adenosine 5'-triphosphate at preganglionic nerve terminals in ciliary ganglia (Bennett & Ho, 1990), has a role in modulating impulse traffic in tonic-firing ciliary neurones.

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