Nitric oxide modulation of calcium-activated potassium channels in postganglionic neurones of avian cultured ciliary ganglia

M. Cetiner & ¹M.R. Bennett

The Neurobiology Laboratory, Department of Physiology, University of Sydney, N.S.W. 2006, Australia

1 A study has been made of the modulation of calcium-activated potassium channels in cultured neurones of avian ciliary ganglia by sodium nitroprusside and L-arginine.

2 Sodium nitroprusside (100 μM) reduced the net outward current by $22 \pm 1\%$ at 4.8 ms (mean $\pm$ s.e.mean) and $25 \pm 1\%$ at 350 ms during a test depolarization to +40 mV from a holding potential of –40 mV. The outward current remained reduced for the duration of the recording following a single application of sodium nitroprusside. These effects did not occur if the influx of calcium ions was first blocked with Cd^{2+} (500 μM). Application of ferrocyanide (100 μM) reduced the net outward current by only $6 \pm 3\%$ at 350 ms during a test depolarization to +40 mV.

3 L-Arginine (270 μM) reduced the net outward current on average by $19 \pm 2\%$ at 4.8 ms and $22 \pm 2\%$ at 350 ms during a test depolarization to +40 mV. The current remained in this reduced state for the duration of the recording following a single application of L-arginine. These effects were reduced to $11 \pm 1\%$ at 4.8 ms and $11 \pm 2\%$ at 350 ms in the presence of N^ω-nitro-L-arginine methyl ester (L-NAME, 100 μM).

4 In order to alleviate the dependence of calcium-activated potassium channels ($I_{\text{k(Ca)}}$) on the inward flux of calcium ions, the patch-clamp pipettes were filled with a solution containing 100 μM CaCl_2 , and the Ca^{2+} in the bathing solution was replaced with EGTA. Under these conditions sodium nitroprusside reduced the total outward current during a depolarizing pulse of +40 mV by $9 \pm 1\%$ at 4.8 ms and by $36 \pm 3\%$ at 350 ms. L-Arginine (270 μM) reduced this current under the same conditions by $9 \pm 1\%$ at 4.8 ms and by $35 \pm 2\%$ at 350 ms.

5 Calcium-activated potassium currents were sensitive to apamin (50 nM), as this reduced the outward current by $23 \pm 3\%$ at 350 ms when a high calcium-containing pipette was used during a depolarizing command to +40 mV. L-Arginine still decreased the outward current in the presence of apamin (50 nM), by $5 \pm 1\%$ at 4.8 ms and by $19 \pm 2\%$ at 350 ms, indicating that L-arginine could reduce an apamin-insensitive $I_{\text{k(Ca)}}$.

6 Calcium-activated potassium currents were also sensitive to charybdotoxin (10 nM), as this reduced the outward current by $34 \pm 4\%$ at 350 ms when a high calcium-containing pipette was used during a depolarizing command to +40 mV. L-Arginine still decreased the outward current in the presence of charybdotoxin, by $6 \pm 1\%$ at 4.8 ms and $12 \pm 4\%$ at 350 ms, showing that L-arginine could reduce a charybdotoxin-insensitive $I_{\text{k(Ca)}}$.

7 The present results indicate that NO-synthase in ciliary ganglia can modulate $I_{\text{k(Ca)}}$ by a method which is independent of the action of NO on the calcium channels. The $I_{\text{k(Ca)}}$ is decreased significantly at 4.8 ms into a depolarizing pulse, at a time that would decrease the rate of repolarization of the action potential. $I_{\text{k(Ca)}}$ is also reduced at longer times (350 ms), indicating an affect on the inactivating process.

Keywords: Nitric oxide; calcium-activated potassium channels; postganglionic neurones; avian cultured ciliary ganglia

Introduction

Nitric oxide (NO) acts as a transmitter in the nervous system as well as a possible retrograde messenger from the neurones to the synapses which impinge upon them (Bredt & Snyder, 1992). For example, the inhibitory junction potential in the gastrointestinal tract (Bennett *et al.*, 1966) is likely to be due to the release of NO from the enteric inhibitory neurones in the gut (for a review see Rand, 1992). In the hippocampus, long-term potentiation (LTP) of transmission at the synapses between Schaffer collaterals and CA1 pyramidal neurones may be due in part to the action of NO on nerve terminals after its synthesis in the pyramidal neurones consequent on the influx of calcium ions through NMDA receptors and the activation of NO-synthase (Böhme *et al.*, 1991; for a review see Bredt & Snyder, 1992). It has recently been discovered that NO-synthase is present in the avian ciliary ganglion (Scott *et al.*, 1992) and that the ganglion possesses a form of long-term potentiation (Scott & Bennett, 1992; 1993a; for a

review see Kuba & Kumamoto, 1990). Because the ganglion has giant calyciform nerve terminals (de Lorenzo, 1960), it offers an ideal preparation for the analysis of both the mechanisms of LTP and also how this might be initiated, maintained or otherwise modulated by NO.

One way in which LTP might be initiated involves a decrease in current through potassium channels that participate in the repolarization of the action potential; such a decrease has the effect of increasing the duration of the action potential and so extends the duration of calcium influx in the nerve terminal, thus increasing transmitter release (Klein *et al.*, 1982). Over-expression of potassium channels has been shown to lead to an increase in the rate of repolarization of the action potential in nerve terminals, with a consequent decrease in the duration of the action potential and in calcium influx, so decreasing transmitter release (Kaang *et al.*, 1992). Repolarization of the action potential is in part due to the opening of fast calcium-activated potassium channels (I_{c}) in sympathetic neurones (Lancaster & Pennefather, 1987; Marsh & Brown, 1991) and in the hip-

¹ Author for correspondence.

pocampus (Lancaster & Adams, 1986; Storm, 1987; Yoshida *et al.*, 1991). In chick ciliary ganglion neurones, Dryer *et al.* (1991) showed that $I_{K(Ca)}$ contributes to the late phase of spike repolarization and after-hyperpolarization and by blocking this channel activity they showed that the action potential duration was broadened. In the present work a study has been made of the effects of NO on calcium-activated potassium channels with the idea that if $I_{K(Ca)}$ is blocked by NO this may form the basis for the induction of enhanced transmitter release observed during LTP.

Methods

Tissue culture

Ciliary ganglia were dissected from 9–11 day old chick embryos in Hanks Balanced Salt Solution and then incubated for 15–20 min at 37°C in 1 ml of Ca^{2+} , Mg^{2+} -free Hanks Balanced Salt Solution containing 0.3% bovine serum albumen (BSA). Following the incubation period, the cells were dissociated in the incubation media by repeated (6–8) trituration through a Pasteur pipette, and the remaining clumps of ganglia tissue were allowed to settle to the bottom. The supernatant containing the cell suspension was then removed and stored aside. Then 1 ml of 'Ciliary ganglion medium (CGM)' containing Dulbecco's Modified Eagles Media (DMEM, ICN Biomedicals) supplemented with 2 mM glutamine, 1.00 μml^{-1} penicillin, 50 mg ml^{-1} streptomycin, 10% heat-inactivated horse serum (CSL) and 2% chick embryo extract was added to the remaining ganglia tissue and triturated again (6–8 times) through the Pasteur pipette. This procedure of trituration was repeated 3 more times in CGM giving a single-cell suspension in 5 ml. If required, this suspension was further diluted with CGM to give a concen-

tration of 1 ganglia per well when plated onto 13 mm glass coverslips previously coated with poly-L-lysine in 24-well plates (ICN Biochemicals) and the culture maintained at 37°C in a 5% CO_2 in air water-saturated atmosphere until required. The neurones were used for whole-cell recording within 6 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

Figure 1 (a) Shows set of voltage-clamp currents for a ciliary neurone during command pulses of 470 ms duration to the different levels indicated by the rectangular traces above; the holding potential (Vh) 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 The effect of sodium nitroprusside (100 μM) on the current amplitude during voltage-clamp of the ciliary neurone soma. (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration (indicated by the rectangular trace) to +40 mV from a Vh of -40 mV both before (C) and after sodium nitroprusside (SNP) application. (b) Shows the current-voltage relation before (O) and after sodium nitroprusside (●) application; the currents were measured at 350 ms into the command potential. (c) Shows the effect of SNP on the voltage-clamp current for the same condition as in (a), on an expanded time scale.

capillary tubing and had resistances of 3–4 M Ω when filled with internal solution (Hamill *et al.*, 1981). For the whole-cell recording described 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 7.2–7.3. The bathing solution consisted of (mM): NaCl 140, KCl 5, CaCl₂ 2, MgCl₂ 2, glucose 10, HEPES 10 with NaOH added to give a pH of 7.2–7.3. Variations on these solutions involved addition of EGTA to the pipette or bath solution, addition of CdCl₂ to the bath solution or omission of CaCl₂ from the bath solution. Such changes are noted in the appropriate legends. All experiments were at room temperature (18–21°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 were 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, preventing the reforming of the membrane was maintained throughout 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 1 kHz were sampled at 1.67 kHz.

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.

Figure 3 The effect of sodium nitroprusside (100 μ M) on the delayed rectifier current (I_k). (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to +40 mV from a Vh of -40 mV both before (C) and after sodium nitroprusside (SNP) application. (b) Shows the current-voltage relation before (○) and after sodium nitroprusside (●) application; the currents were measured at 350 ms into the command potential. There was 500 μ M Cd²⁺ in a calcium-free bath and EGTA (10 mM) substituted for KCl (10 mM) in the pipette solution.

Drug application

Drugs which were dissolved in recording media were applied by pressure ejection (2–7 kPa) from micropipettes with tip diameters of about 10 μ m. These pipettes were positioned about 20 to 50 μ m from the neurone cell body during a puff application and then removed. The neurones were required to show stable current magnitudes for at least 20 s before the application of drug, otherwise the results were rejected.

For recording drug effects two methods were used. The first method involved evoking currents by applying a command potential of +40 mV from a holding potential of -40 mV. During repetitive stimulation of this nature, drugs were applied as described above and their effect was determined by calculating the difference in current before and after the drug application. The second method involved recording from a different neurone to produce current versus voltage graphs by applying depolarizing command potentials over a range of voltages.

Figure 4 Separation of the total 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 evoked by command potentials of 360 ms duration to +40 mV from a Vh of -40 mV before (C) and during the application of 500 μ M Cd²⁺ (Cd); the middle trace representing $I_{k(Ca)}$ (C-Cd) is the difference between the currents before and after Cd²⁺. (b) Shows the current-voltage relation for the current (I_k) remaining during the application of Cd²⁺ (●), as well as the current-voltage relation for the current ($I_{k(Ca)}$) (○) due to subtracting the current (I_k) remaining after Cd²⁺ from the control current (not shown). (c) Shows the effect of sodium nitroprusside (100 μ M) on $I_{k(Ca)}$ after the I_k component has been subtracted from the total outward current from Figure 2a.

Drugs

Drugs obtained from Sigma were: L-arginine, N^ω-nitro-L-arginine methyl ester (L-NAME) and apamin. Sodium nitroprusside was obtained from Ajax Chemicals, potassium ferrocyanide from B.D.H. Chemicals and charybdotoxin from NPS Pharmaceuticals (formerly Natural Product Sciences, Inc.), Salt Lake City, UTAH 84108, U.S.A.

Statistics

Data are expressed as mean $\pm$ s.e.mean and n represents the number of experiments. The significance of the difference between the n pairs of observations made before and after application of a drug was calculated by Student's t test. P values of 0.05 or less were considered to represent significant differences.

Figure 5 The effect of L-arginine ($270 \mu\text{M}$) on the current amplitude during voltage-clamp of the ciliary neurone soma. (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to $+40 \text{ mV}$ from a V_h of -40 mV both before (C) and after L-arginine (L-Arg) application. (b) Shows the current-voltage relation before (○) and after L-arginine (●) application; the currents were measured at 350 ms into the command potential. (c) Shows the effect of L-arginine on the voltage-clamp current for the same condition as in (a), on an expanded time scale.

Results

Depolarizing voltage-command steps of 470 ms duration from holding potential (V_h) of -40 mV gave outward currents of the kind shown in Figure 1a in all ciliary neurones studied ($n = 15$). The current increases in amplitude up to depolarizations of 80 mV ; thereafter the current declines to reach a minimum at about 110 mV from which it increases again (Figure 1b). This N-shaped current-voltage curve is due to the decrease in the calcium-activated potassium outward current ($I_{\text{K(Ca)}}$) that occurs for depolarizing commands that approach the calcium equilibrium potential (see for example Bennett *et al.*, 1992). The further increase in current at higher depolarizations is primarily due to the delayed rectifier (I_{K}) (Bennett *et al.*, 1991).

Application of sodium nitroprusside (SNP; $100 \mu\text{M}$) from a puffer pipette decreased the amplitude of the outward current during command steps over a wide range of voltages (Figure 2b); this reduction amounted to $22 \pm 1\%$ ($n = 32$) at 4.8 ms and $25 \pm 1\%$ at 350 ms for a command voltage of $+40 \text{ mV}$; both of these changes were very significant (Figure 2a,c). In order to check that SNP was not having an effect through the action of ferrocyanide, this was applied to the ganglion through a puffer pipette. Ferrocyanide ($100 \mu\text{M}$) decreased the amplitude of the current by only $6 \pm 3\%$ at 350 ms ($n = 8$; recording not shown), indicating that it was predominantly NO that modulated the channel activity.

From Figure 1 we know that the total current represented in Figure 2 is due primarily to two types of current, namely $I_{\text{K(Ca)}}$ and the I_{K} . The effect of SNP ($100 \mu\text{M}$) on I_{K} was investigated by blocking the influx of calcium ions with Cd^{2+} ($500 \mu\text{M}$) in the recording bath, so blocking $I_{\text{K(Ca)}}$ (Bennett *et al.*, 1991). Under these conditions, the application of SNP did not affect the size of the current at any command potential (Figure 3b); at a command potential of $+40 \text{ mV}$ SNP decreased the I_{K} by $1 \pm 3\%$ at 350 ms ($n = 7$; Figure 3a),

Figure 6 The effect of L-arginine ($270 \mu\text{M}$) in the presence of N^ω-nitro-L-arginine methyl ester (L-NAME, $100 \mu\text{M}$) on the current amplitude, during voltage-clamp of the ciliary neurone soma. (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to $+40 \text{ mV}$ from a V_h of -40 mV both before (C) and after L-arginine (L-Arg + L-NAME) application. (b) Shows the current-voltage relation before (○) and after L-arginine (●) application; the currents were measured at 350 ms into the command potential. L-NAME ($100 \mu\text{M}$) was added to the bath.

which was not significant. Thus the effect of SNP on the total outward current is due to its effect on $I_{k(Ca)}$.

The proportion of the total outward current due to I_k can be determined by blocking $I_{k(Ca)}$ with $500 \mu\text{M Cd}^{2+}$. Figure 4a shows that at a command potential of $+40 \text{ mV}$, the I_k amounted to about 30% of the outward current at 350 ms; subtraction of this gives a measure of the large $I_{k(Ca)}$ current. According to this analysis $I_{k(Ca)}$ provided most of the outward current in the voltage-range from 0 mV to $+120 \text{ mV}$ (Figure 4b). In order to obtain a measure of the effect of SNP on $I_{k(Ca)}$ at a command potential of $+40 \text{ mV}$, the following procedure was adopted: the I_k measured in the experiment of

Figure 4a was subtracted from both the control and SNP-treated currents given in the experiment of Figure 2a; the resulting currents are shown in Figure 4c. When the average I_k over 4 experiments was subtracted from the average control of SNP treated cells in 32 experiments, the decrease in $I_{k(Ca)}$ due to SNP was 45% at 350 ms.

L-Arginine ($270 \mu\text{M}$) was applied to voltage-clamped ganglion cells in order to test whether endogenous nitric oxide was being produced by the nitric oxide synthase system present in the ganglion (Figure 5). The current-voltage curve for different command voltages showed that L-arginine reduced the current over the entire range of depolarizing

Figure 7 The effect of sodium nitroprusside ($100 \mu\text{M}$) on $I_{k(Ca)}$ during voltage-clamp of the ciliary neurone soma, under the conditions of no calcium currents. (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to $+40 \text{ mV}$ from a V_h of -40 mV both before (C) and during sodium nitroprusside (SNP) application. (b) Shows the current-voltage relation before (○) and after sodium nitroprusside (●) application; the currents were measured at 350 ms into the command potential. (c) Shows the effect of SNP on the voltage-clamp current for the same condition as in (a), on an expanded time scale. There was no Ca^{2+} in the bath solution, with 10 mM EGTA substituted for 10 mM NaCl in the bath; $100 \mu\text{M CaCl}_2$ was added to the patch-pipette solution.

Figure 8 The effect of L-arginine ($270 \mu\text{M}$) on $I_{k(Ca)}$ during voltage-clamp of the ciliary neurone soma, under the conditions of no calcium currents. (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to $+40 \text{ mV}$ from a V_h of -40 mV both before (C) and during L-arginine (L-Arg) application. (b) Shows the current-voltage relation before (○) and after L-arginine (●) application; the currents were measured at 350 ms into the command potential. (c) Shows the effect of L-arginine on the voltage-clamp current for the same condition as in (a), on an expanded time scale. There was no Ca^{2+} in the bath solution with 10 mM EGTA substituted for 10 mM NaCl in the bath; $100 \mu\text{M CaCl}_2$ was added to the patch-pipette solution.

command voltages (Figure 5b). At a command voltage of +40 mV the current was significantly reduced by $19 \pm 2\%$ at 4.8 ms ($n = 15$; Figure 5c) and $22 \pm 2\%$ at 350 ms (Figure 5a). L-NAME ($100 \mu\text{M}$), a competitive inhibitor of NO-synthase was added to the bath in order to show that L-arginine was working via NO-synthase. Under these conditions L-arginine decreased the total outward current by a reduced amount, namely $11 \pm 1\%$ at 4.8 ms and $11 \pm 2\%$ at 350 ms ($n = 12$; Figure 6).

It has already been shown that NO can decrease the transient and sustained calcium currents in this ganglion (Khurana & Bennett, 1993), so the decrease in $I_{\text{k(Ca)}}$ in the presence of NO may be due to the decrease in calcium influx. In order to test whether the $I_{\text{k(Ca)}}$ could be directly modulated

by NO, a calcium-free solution with 10 mM EGTA was added to the bath to prevent the influx of calcium. The patch-clamp pipette was then filled with a solution that contained $100 \mu\text{M}$ CaCl_2 to activate $I_{\text{k(Ca)}}$ directly. Under these conditions a large $I_{\text{k(Ca)}}$ could be generated, as much of the outward current could be blocked with both apamin and charybdotoxin (CTx) (see below). When either SNP ($100 \mu\text{M}$) or L-arginine ($270 \mu\text{M}$) were applied, the outward current was decreased over the entire range of positive command-potentials studied (Figures 7b and 8b). At a command potential of +40 mV SNP significantly reduced the current by $9 \pm 1\%$ at 4.8 ms and by $36 \pm 3\%$ at 350 ms ($n = 6$; Figure 7a,c) and L-arginine reduced the current by $9 \pm 1\%$ at 4.8 ms and by $35 \pm 2\%$ at 470 ms ($n = 27$; Figure 8a,c).

Figure 9 The effect of L-arginine ($270 \mu\text{M}$) on $I_{\text{k(Ca)}}$ in the presence of apamin (50 nM) during voltage-clamp of the ciliary neurone soma, under the conditions of no calcium currents. (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to +40 mV from a Vh of -40 mV both before (C) and during apamin (A) application. (b) Shows the current-voltage currents evoked by command potentials of 360 ms duration to +40 mV from a Vh of -40 mV both before (C) and during L-arginine (L-Arg) application, in the presence of apamin (50 nM). (c) Shows the current-voltage relation before (○) and after L-arginine (●) application in the presence of apamin (50 nM); the currents were measured at 350 ms into the command potential. There was no Ca^{2+} in the bath solution with 10 mM EGTA substituted for 10 mM NaCl in the bath; $100 \mu\text{M}$ CaCl_2 was added to the patch-pipette solution.

Figure 10 The effect of L-arginine ($270 \mu\text{M}$) on $I_{\text{k(Ca)}}$ in the presence of charybdotoxin (10 nM) during voltage-clamp of the ciliary neurone soma, under the conditions of no calcium currents. (a) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to +40 mV from a Vh of -40 mV both before (C) and during charybdotoxin (CTx) application. (b) Shows the voltage-clamp currents evoked by command potentials of 360 ms duration to +40 mV from a Vh of -40 mV both before (C) and during L-arginine (L-Arg) application, in the presence of charybdotoxin (10 nM). (c) Shows the current-voltage relation before (○) and after L-arginine (●) application in the presence of charybdotoxin (10 nM); the currents were measured at 350 ms into the command potential. There was no Ca^{2+} in the bath solution with 10 mM EGTA substituted for 10 mM NaCl in the bath; $100 \mu\text{M}$ CaCl_2 was added to the patch-pipette solution.

In order to determine if the action of NO on $I_{K(Ca)}$ was specific to the apamin-sensitive (SK type) low conductance $I_{K(Ca)}$ (Latorre *et al.*, 1989; Dreyer, 1990) and/or to the charybdotoxin (CTx)-sensitive (BK or maxi-K type) high conductance $I_{K(Ca)}$ (Latorre *et al.*, 1989; Dreyer, 1990), these substances were used to block the channels and the effects of L-arginine then determined. At a command potential of +40 mV, application of apamin (50 nM) significantly reduced the current by $23 \pm 3\%$ ($n = 7$) at 350 ms indicating the presence of SK type $I_{K(Ca)}$ (Figure 9a). Application of L-arginine (270 μ M) under these conditions still significantly reduced the current by $5 \pm 1\%$ at 4.8 ms ($n = 11$) and by $19 \pm 2\%$ at 350 ms (Figure 9b). This effect of L-arginine occurred over the entire range of positive command potentials studied (Figure 9c). Application of CTx (10 nM) also significantly reduced the current in response to a command potential of +40 mV by $34 \pm 4\%$ at 350 ms ($n = 7$; Figure 10a). In the presence of 10 nM CTx, application of L-arginine (270 μ M) further significantly reduced the current by $6 \pm 1\%$ at 4.8 ms ($n = 8$) and by $12 \pm 4\%$ at 350 ms (Figure 10b). The reduction of current in the presence of CTx also occurred over the entire range of positive command potentials studied (Figure 10c). These results imply that NO acts on both the apamin-insensitive and the CTx-insensitive $I_{K(Ca)}$.

Discussion

The ciliary ganglion is known to possess a number of different potassium channel types, which are likely to participate in both the repolarization and after-hyperpolarization phases of the action potential. In the neurone soma there are delayed rectifier channels, calcium-activated potassium channels, inward rectifier potassium channels, cholinergic agonist activated channels, fast-transient potassium channels, and sodium-activated potassium channels (Bennett *et al.*, 1991). A number of different potassium channels have also been identified in the calyciform nerve terminal: these include delayed rectifier channels, inward rectifier channels and calcium-activated potassium channels (Bennett & Ho, 1991; Fletcher & Chiappinelli, 1992). The identity of the type of $I_{K(Ca)}$ is not clear, although an $I_{K(Ca)}$ is blocked by tetraethylammonium ions (TEA; Bennett & Ho, 1992), and it is known that the fast $I_{K(Ca)}$, I_C , which contributes to the repolarization of the action potential is very sensitive to TEA in autonomic ganglia (Lancaster & Pennefather, 1987; Marsh & Brown, 1991). The $I_{K(Ca)}$ studied in the present work consisted of a charybdotoxin-sensitive component and an apamin-sensitive component. The former is likely to be the I_C current (Dryer *et al.*, 1991). Apamin is known to suppress the $I_{K(Ca)}$ involved in the after-hyperpolarization of the action potential but not the $I_{K(Ca)}$ involved in the repolarization of the action potential in autonomic neurones (Tanaka *et al.*, 1986; Kawai & Watanabe, 1986; Dryer *et al.*, 1991). Taken together with the present observations that show NO can depress both apamin and charybdotoxin-sensitive $I_{K(Ca)}$ at times comparable to that of the repolarization phase of the action potential at about 5 ms (Dryer *et al.*, 1991), it seems likely that both the I_C current involved in the repolarization of the action potential as well as the apamin-sensitive current involved in the after-hyperpolarization are depressed.

There is considerable evidence to suggest that changes in I_C occur in nerve terminals under physiological conditions which lead to changes in transmitter release. For example,

somatostatin inhibits transmitter release from pituitary cells by stimulating a maxi-K channel, probably responsible for the I_C current, through a protein dephosphorylation mechanism, leading to an increased rate of repolarization during the action potential and so a decrease in calcium entry (White *et al.*, 1991). In *Hermisenda*, behavioural changes elicited by associative conditioning have focussed on the reduction of I_C in the nerve terminal that are brought about by the phosphorylation of a G protein (Nelson *et al.*, 1990). At the amphibian neuromuscular junction charybdotoxin-sensitive channels normally decrease the duration of the terminal action potential and thereby determine the extent of calcium entry into the terminal, and therefore the amount of transmitter released (Robitaille & Charlton, 1992). This process is facilitated by the I_C channels being placed in the vicinity of the high density of calcium-channels that are strategically positioned at the release sites (Roberts *et al.*, 1990; Gola *et al.*, 1990; Robitaille & Charlton, 1992).

NO blocks high-voltage threshold calcium channels in the ciliary ganglion (Khurana & Bennett, 1993) as well as blocking calcium influx in smooth muscle (Magliola & Jones, 1990; Clapp & Gurney, 1991). Any action of NO in directly depressing I_C during the time of the repolarization of the action potential at about 5 ms (Dryer *et al.*, 1991), and therefore potentially increasing the duration of nerve terminal action potentials, calcium influx and transmitter release, may therefore be offset by NO directly depressing calcium influx. However, it is not clear at present whether the high-voltage calcium channels that are blocked by NO are the calcium channels that are strategically placed at the release sites of nerve terminals and are involved in transmitter release by the nerve impulse. These channels are different from the high-voltage threshold L or N-type calcium channels (Fox *et al.*, 1987) at least in the case of the calyciform nerve terminal of the avian ciliary ganglion (Stanley, 1991).

In the present work, the calcium-dependent potassium currents were depressed to a much greater extent by L-arginine at 4.8 ms in normal calcium, than by L-arginine at this time when a calcium-free solution was used with high-calcium containing pipettes. The larger depression in the former case is probably due to NO blocking both calcium entry as well as having a direct effect on the activation of the potassium channels. At 350 ms the calcium-dependent potassium currents are substantially reduced by L-arginine independent of whether calcium enters these cells through the calcium channels or not. Inactivation of the potassium channels therefore appears to be especially sensitive to NO.

The mechanism of action of NO in the ganglion, by which it decreases $I_{K(Ca)}$, may in part involve elevation of guanosine 3':5'-cyclic monophosphate (cyclic GMP). Addition of 8-Br-cyclic GMP to the ganglion enhances synaptic transmission (Scott & Bennett, 1993b) indicating a possible role for endogenous guanylate cyclase in the action of NO in increasing the efficacy of transmission in the ganglion (Scott & Bennett, 1993b). NO can elevate levels of cyclic GMP in smooth muscle cells to produce a hyperpolarization that closes voltage-sensitive calcium channels (Cornwell & Lincoln, 1989). It seems unlikely that this occurs in the ciliary ganglion, as NO only seems to have a depressing effect on potassium channels and this would not lead to hyperpolarization of the terminals with a consequent closing of voltage-sensitive calcium channels. It remains to be determined however whether the effects of NO on the potassium channels of the ciliary ganglion soma are the same as those on potassium channels of the calyciform nerve terminals.

References

- BENNETT, M.R. & HO, S. (1991). Probabilistic secretion of quanta from nerve terminals in avian ciliary ganglia modulated by adenosine. *J. Physiol.*, **440**, 513–527.
- BENNETT, M.R. & HO, S. (1992). Adenosine modulation of potassium currents in the preganglionic nerve terminals of avian ciliary ganglia. *Neurosci. Lett.*, **137**, 41–44.

- BENNETT, M.R., BURNSTOCK, G. & HOLMAN, M.E. (1966). Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol.*, **182**, 541–558.
- BENNETT, M.R., KERR, R. & KHURANA, G. (1992). Adenosine modulation of calcium currents in postganglionic neurones of avian cultured ciliary ganglia. *Br. J. Pharmacol.*, **106**, 25–32.
- BENNETT, M.R., KERR, R. & NICHOL, K. (1991). Adenosine modulation of potassium currents in the postganglionic neurones of cultured avian ciliary ganglia. *Br. J. Pharmacol.*, **104**, 459–465.
- BÖHME, C.A., BON, C., STUTZMANN, J.M., DOBLE, A. & BLANCHARD, J.C. (1991). Possible involvement of nitric oxide in long-term potentiation. *Eur. J. Pharmacol.*, **199**, 379–381.
- BREDT, D.S. & SNYDER, S.H. (1992). Nitric oxide, a novel neuronal messenger. *Neuron*, **8**, 3–11.
- CLAPP, L.H. & GURNEY, A.M. (1991). Modulation of calcium movements by nitroprusside in isolated vascular smooth muscle cells. *Pflügers Arch.*, **418**, 462–470.
- CORNWELL, T.L. & LINCOLN, T.M. (1989). Regulation of intracellular Ca^{2+} levels in cultured vascular smooth muscle cells. *J. Biol. Chem.*, **264**, 1146–1155.
- DE LORENZO, A.J. (1960). The fine structure of synapses in the ciliary ganglion of the chick. *J. Biophys. Biochem. Cytol.*, **7**, 31–36.
- DREYER, D. (1990). Peptide toxins and potassium channels. *Rev. Physiol. Biochem. Pharmacol.*, **115**, 93–136.
- DRYER, S.E., DUORADO, M.M. & WISGIRDA, M.E. (1991). Characteristics of multiple Ca^{2+} -activated K^{+} channels in acutely dissociated chick ciliary-ganglion neurones. *J. Physiol.*, **443**, 601–627.
- FENWICK, E.M., MARTY, A. & NEHER, E. (1982). A patch-clamp study of bovine chromaffin cells and their sensitivity to acetylcholine. *J. Physiol.*, **331**, 577–597.
- FLETCHER, G.H. & CHIAPPINELLI, V.A. (1992). An inward rectifier is present in presynaptic nerve terminals in the chick ciliary ganglion. *Brain Res.*, **575**, 103–112.
- FOX, A.P., NOWYCKY, M.C. & TSIEN, R.W. (1987). Kinetic and pharmacological properties distinguishing three types of calcium currents in the chick sensory neurones. *J. Physiol.*, **373**, 149–172.
- GOLA, M., DUCREUX, C. & CHAGNEUX, H. (1990). Ca^{2+} -activated K^{+} current involvement in neuronal function revealed by *in situ* single-channel analysis in Helix neurones. *J. Physiol.*, **420**, 73–109.
- HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, G. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.
- KAANG, B.K., PFAFFINGER, P.J., GRANT, S.G.N., KANDEL, E.R. & FURUKAWA, Y. (1992). Overexpression of an Aplysia Shaker K^{+} channel gene modifies the electrical properties and synaptic efficacy of identified Aplysia neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 1133–1137.
- KAWAI, T. & WATANABE, M. (1986). Blockade of Ca -activated K conductance by apamin in rat sympathetic neurones. *Br. J. Pharmacol.*, **87**, 225–232.
- KHURANA, G. & BENNETT, M.R. (1993). Nitric oxide and arachidonic acid modulation of calcium currents in postganglionic neurones of cultured avian ciliary ganglia. *Br. J. Pharmacol.*, **109**, 480–485.
- KLEIN, M., CAMARDO, J. & KANDEL, E.R. (1982). Serotonin modulates a specific potassium current in the sensory neurons that show presynaptic facilitation in Aplysia. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 5713–5717.
- KUBA, K. & KUMAMOTO, E. (1990). Long-term potentiation in vertebrate synapses: a variety of cascades with common sub-processes. *Prog. Neurobiol.*, **34**, 197–269.
- LANCASTER, B. & ADAMS, P.R. (1986). Calcium-dependent current generating the afterhyperpolarization of hippocampal neurons. *J. Neurophysiol.*, **55**, 1268–1282.
- LANCASTER, B. & PENNEFATHER, P. (1987). Potassium currents evoked by brief depolarizations in bull-frog sympathetic ganglion cells. *J. Physiol.*, **387**, 519–548.
- LATORRE, R., OBERHAUSER, A., LABARCA, P. & ALVAREZ, O. (1989). Varieties of calcium-activated potassium channels. *Annu. Rev. Physiol.*, **51**, 385–399.
- MAGLIOLA, L. & JONES, A.W. (1990). Sodium nitroprusside alters Ca^{2+} flux components and Ca^{2+} -dependent fluxes of K^{+} and Cl^{-} in rat aorta. *J. Physiol.*, **421**, 411–424.
- MARSH, S.H. & BROWN, D.A. (1991). Potassium currents contributing to action potential repolarization in dissociated cultured rat superior cervical sympathetic neurones. *Neurosci. Lett.*, **433**, 298–302.
- NELSON, T.J., COLLIN, C. & ALKON, D. (1990). Isolation of a G protein that is modified by learning and reduces potassium currents in *Hermisenda*. *Science*, **247**, 1479–1483.
- RAND, M.J. (1992). New perspectives in neurotransmission: nitric oxide, the mediator of nitrergic transmission. *Proc. Aust. Physiol. Pharmacol. Soc.*, **23**, 1–16.
- ROBERTS, W.M., JACOBS, R.A. & HUDSPETH, A.J. (1990). Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J. Neurosci.*, **10**, 3664–3684.
- ROBITAILLE, R. & CHARLTON, M.P. (1992). Presynaptic calcium signals and transmitter release are modulated by calcium-activated potassium channels. *J. Neurosci.*, **12**, 297–305.
- SCOTT, T.R.D. & BENNETT, M.R. (1992). Long-term potentiation of chemical transmission in the avian ciliary ganglion. *Proc. Aust. Physiol. Pharmacol. Soc.*, **23**, 182P.
- SCOTT, T.R.D. & BENNETT, M.R. (1993a). The effect of ions and second messengers on long-term potentiation of chemical transmission in avian ciliary ganglia. *Br. J. Pharmacol.*, (in press).
- SCOTT, T.R.D. & BENNETT, M.R. (1993b). The role of cGMP in the persistent enhancement of the efficacy of synaptic transmission in the chick ciliary ganglion. *Proc. Aust. Neurosci. Soc.*, **4**, ORA 17.5.
- SCOTT, T.R.D., NICHOL, K.A., CHAN, N. & BENNETT, M.R. (1992). Nitric oxide synthase localization in autonomic ciliary ganglia and its participation in long-term potentiation. *Proc. Aust. Physiol. Pharmacol. Soc.*, **23**, 191P.
- STANLEY, E.F. (1991). Single calcium channels as a cholinergic presynaptic nerve terminal. *Neuron*, **7**, 585–591.
- STORM, J.F. (1987). Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. *J. Physiol.*, **385**, 733–759.
- TANAKA, K., MINOTA, S., KUBA, K., KOYANO, K. & ABE, T. (1986). Differential effects of apamin on Ca^{2+} -activated K currents in bullfrog sympathetic ganglion cells. *Neurosci. Lett.*, **69**, 233–238.
- WHITE, R.E., SCHONBRUNN, A. & ARMSTRONG, D.L. (1991). Somatostatin stimulates Ca^{2+} -activated K^{+} channels through protein dephosphorylation. *Nature*, **351**, 570–573.
- YOSHIDA, A., ODA, M. & IKEMOTO, Y. (1991). Kinetics of the Ca^{2+} -activated K^{+} channel in rat hippocampal neurons. *J. Physiol.*, **41**, 297–315.

(Received March 2, 1993)

Revised June 9, 1993

Accepted July 7, 1993)