Br. J. Pharmacol. (1993), **109**, 480–485

Nitric oxide and arachidonic acid modulation of calcium currents in postganglionic neurones of avian cultured ciliary ganglia

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1 A study has been made of the modulation of high-voltage activated transient and sustained calcium currents in cultured neurones of avian ciliary ganglia by nitric oxide (NO) and arachidonic acid.

2 Sodium nitroprusside (100 μM) reduced the transient calcium current (I_{Ca}) on average by 31% and the sustained I_{Ca} by 32% during a test depolarization to +20 mV from a holding potential of −100 mV. This reduction was maintained for at least 30 min following a single application of sodium nitroprusside.

3 L-Arginine (270 μM) reduced the transient I_{Ca} on average by 28% and the sustained I_{Ca} by 22% and these effects were prevented by the presence of the NO-synthase competitive blocker N^G-nitro-L-arginine methylester (L-NAME; 100 μM) in the bathing solution.

4 Arachidonic acid (50 μM) reduced the transient I_{Ca} on average by 28% and the sustained I_{Ca} by 33%. When added together, arachidonic acid (50 μM) and L-arginine (270 μM) produced the same effects as arachidonic acid alone.

5 Blocking the conversion of arachidonic acid to prostaglandins by addition of indomethacin (20 μM) to the bathing solution did not prevent the depression of either the transient or the sustained calcium current during application of arachidonic acid (50 μM). The effects of arachidonic acid were also not occluded by L-NAME (100 μM) when present in the bathing solution.

6 Inhibiting the biosynthesis of leukotrienes by applying L-663,536 (MK-886; 3 μM) to the bathing solution prevented the depression of both components of I_{Ca} during application of arachidonic acid (50 μM).

7 These results indicate that endogenous NO and arachidonic acid pathways are present in parasympathetic ciliary neurones, and that both act to depress high-voltage, gated, calcium channel activity.

Keywords: Nitric oxide; arachidonic acid; calcium current; postganglionic neurones; avian ciliary ganglia

Introduction

Nitric oxide synthase (NOS; Bredt & Snyder, 1992) is present in the cell bodies of avian ciliary ganglia and NO enhances transmitter secretion in the ganglion (Scott *et al.*, 1992). One possible mechanism by which NO may modulate transmitter release is through an action on calcium channels. NO can elevate levels of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) in smooth muscle cells to produce a hyperpolarization that closes voltage-sensitive calcium channels (Clapp & Gurney, 1991). NO can also act through cyclic GMP to prevent the noradrenaline-induced increase in cytosolic calcium levels via an inhibition of both calcium influx and intracellular calcium release (Tare *et al.*, 1990). It should also be noted that elevated cyclic GMP levels are known to depress the calcium current through high-voltage-activated calcium channels in acutely isolated hippocampal neurones (Doerner & Alger, 1988).

Ciliary neurones possess high-voltage-activated calcium channels that mediate a rapidly inactivating calcium current and a slowly inactivating calcium current (Bennett *et al.*, 1992), which may be referred to as 'transient' and 'sustained' currents, respectively (Schroeder *et al.*, 1990; Sher & Clementi, 1991). Both transient and sustained calcium currents are reduced by the dihydropyridine antagonist nifedipine as well as by ω-conotoxin (Bennett *et al.*, 1992), so that these currents cannot be assigned to distinct types of high-threshold calcium channels (Fox *et al.*, 1987a,b; for reviews see Scott *et al.*, 1991; Swandulla *et al.*, 1991). In the present work both transient and sustained currents have been recorded from nine to twelve day old chick embryo ciliary neurones and their relative sensitivity to NO determined.

Arachidonic acid has been shown to inhibit calcium current in *Aplysia* sensory neurones (Piomelli *et al.*, 1987) and also in neurones of the chick sympathetic ganglion (Bug *et al.*, 1989) and the guinea-pig hippocampus (Keyser & Alger, 1990). Furthermore, it has recently been demonstrated that arachidonic acid can modulate NO production as the cyclooxygenase pathway of arachidonic acid metabolism gives rise to prostaglandins which can activate the L-arginine:NO pathway (Gray *et al.*, 1991). It was therefore of interest to determine if arachidonic acid or one of its prostaglandin metabolites could modulate the transient or sustained calcium currents in parasympathetic neurones of the chick ciliary ganglion and, if so, whether this was due to an action on the L-arginine:NO pathway.

Methods

Ciliary ganglion cell preparation and recording

Neurones from ciliary ganglia dissected from 9–12 day old white leghorn chick embryos were cultured and whole-cell patch-clamped according to the methods described in Bennett *et al.* (1992). Cells were held at a potential of −100 mV for 4 s prior to depolarization to the test potential. Different ciliary neurones showed the peak currents occurring between 0 and +20 mV, although for any single neurone the peak current in control stimulations occurred consistently at the same voltage. Drug effects were consistent between neurones.

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Solutions

Ciliary neurones were placed in a bathing solution at 21°C which consisted of (in mM): $CaCl_2$ 10, TEACl 5, NaCl 125, glucose 10, HEPES 10, and tetrodotoxin (TTX) 300 nM, with the pH adjusted to 7.3 with NaOH. Pipettes of boro-silicate capillary tubing (resistance 4 to 10 MΩ; Hamill *et al.*, 1981) were filled with a solution which consisted of (in mM): CsCl 100, EGTA 10, $MgCl_2$ 5, HEPES 40, ATP 2 and GTP 0.35, with the pH adjusted to 7.3 with CsOH.

Drugs

Sodium nitroprusside, L-arginine, N^G-nitro-L-arginine methylester (L-NAME), and indomethacin (all from Sigma Chemical Co.) were dissolved in recording medium, while arachidonic acid, oleic acid (Sigma Chemical Co.) and L-663,536 (MK-886; 3-[1(4-chloro-benzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2, 2-dimethylpropanoic acid, the kind gift of Dr A.W. Ford-Hutchinson, Merck Frosst Canada Inc.) were dissolved in dimethylsulphoxide (DMSO; 0.05% v/v concentration). Sodium nitroprusside, L-arginine and arachidonic acid were applied by pressure ejection (2 to 7 kPA) from puffer micropipettes with tip diameters of approximately 10 μm. Puffer pipettes were positioned between 20–50 μm from the cell bodies of ciliary neurones during a puff application and then removed.

Statistics

Data are expressed as mean ± standard error of the mean (s.e.mean) and n gives 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 paired t test. P values of 0.05 or less were considered to represent significant differences.

Results

The puffing of bathing solution onto ciliary neurones produced a small but significant decrease in the transient calcium current (I_{Ca}) of 2.2 ± 2% (mean ± s.e.mean; $n = 11$ neurones; $P > 0.25$ using Student's paired t test) for test depolarization

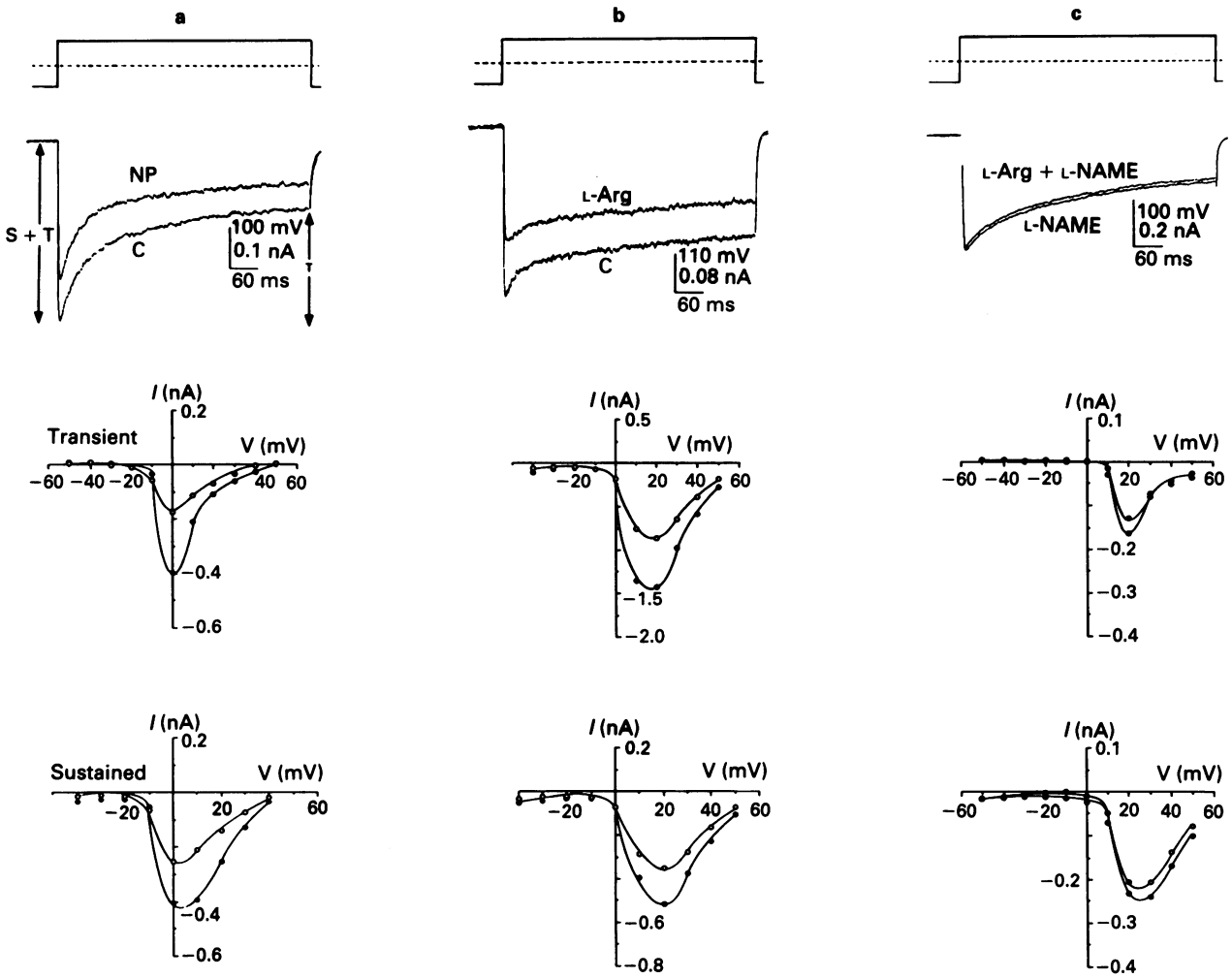

Figure 1 The action of sodium nitroprusside and L-arginine on whole-cell calcium current. The upper panels give the effects of the drugs on the currents generated at + 20 mV from a holding potential of − 100 mV; the broken line indicates the level of − 50 mV. The middle and lower panels give the effects of the drugs on the transient and sustained currents respectively both before (●) and after (○) their puffer application; the voltages are the test potentials. (a) Effect of sodium nitroprusside (100 μM) on whole-cell transient and sustained calcium currents; the upper panel shows the currents evoked both before (C) and after (NP) exposure to sodium nitroprusside; T refers to the transient component of the calcium current and S + T to the sustained plus transient components of the current. (b) Effect of L-arginine (270 μM) on whole-cell transient and sustained calcium currents; in the upper panel are shown the currents evoked both before (C) and after (L-Arg) exposure to L-arginine. (c) Effect of L-arginine (L-Arg; 270 μM) on whole-cell calcium current in the presence of L-N^G-nitroarginine methylester (L-NAME; 100 μM); the upper panel shows the currents evoked in the presence of L-NAME alone (L-NAME) and after exposure to a puff of L-arginine (L-Arg + L-NAME) given 10 s before the observation was made in the presence of L-NAME.

to + 20 mV from a holding potential, V_h, of − 100 mV. A small but significant decrease in the sustained I_{Ca} of 4.9 ± 1% ($n = 11$; $P < 0.002$) occurred under the same conditions.

Nitric oxide

Sodium nitroprusside (100 μM) puffed onto ciliary neurones before a depolarization to + 20 mV from a V_h of − 100 mV, significantly reduced both the transient and sustained components of I_{Ca} (Figure 1a). The transient I_{Ca} was reduced from − 233 ± 37 pA to − 169 ± 33 pA, that is a reduction of 31 ± 8% ($n = 9$; $P < 0.01$), and the sustained I_{Ca} was reduced from − 267 ± 57 pA to − 147 ± 15 pA, that is a reduction of 32 ± 12% ($n = 9$; $P < 0.05$). This depression in I_{Ca} was maintained for at least 30 min following a single puff of sodium nitroprusside. Both sustained and transient I_{Ca} were clearly reduced over the test potential range from − 10 mV to + 30 mV. These results suggest the presence of a nitric oxide-sensitive cyclic GMP system in ciliary neurones that controls the activity of calcium channels.

In order to test whether a nitric oxide synthase system using L-arginine as substrate is likely to be functioning in the ciliary ganglion, the effect of L-arginine puffed onto ganglion cells 10 s before a test depolarization to + 20 mV from a V_h of − 100 mV, was determined (Figure 1b). L-Arginine (270 μM) significantly decreased the transient I_{Ca} from − 355 ± 62 pA to − 250 ± 44 pA, that is a reduction of 28 ± 5% ($n = 16$; $P < 0.01$). L-Arginine also caused a significant reduction in the sustained current, decreasing it by 22 ± 4% ($n = 16$; $P < 0.001$) from − 315 ± 27 pA to − 241 ± 23 pA. The reduction in both components of I_{Ca} occurred over the test potential range from 0 mV to + 50 mV. These effects of

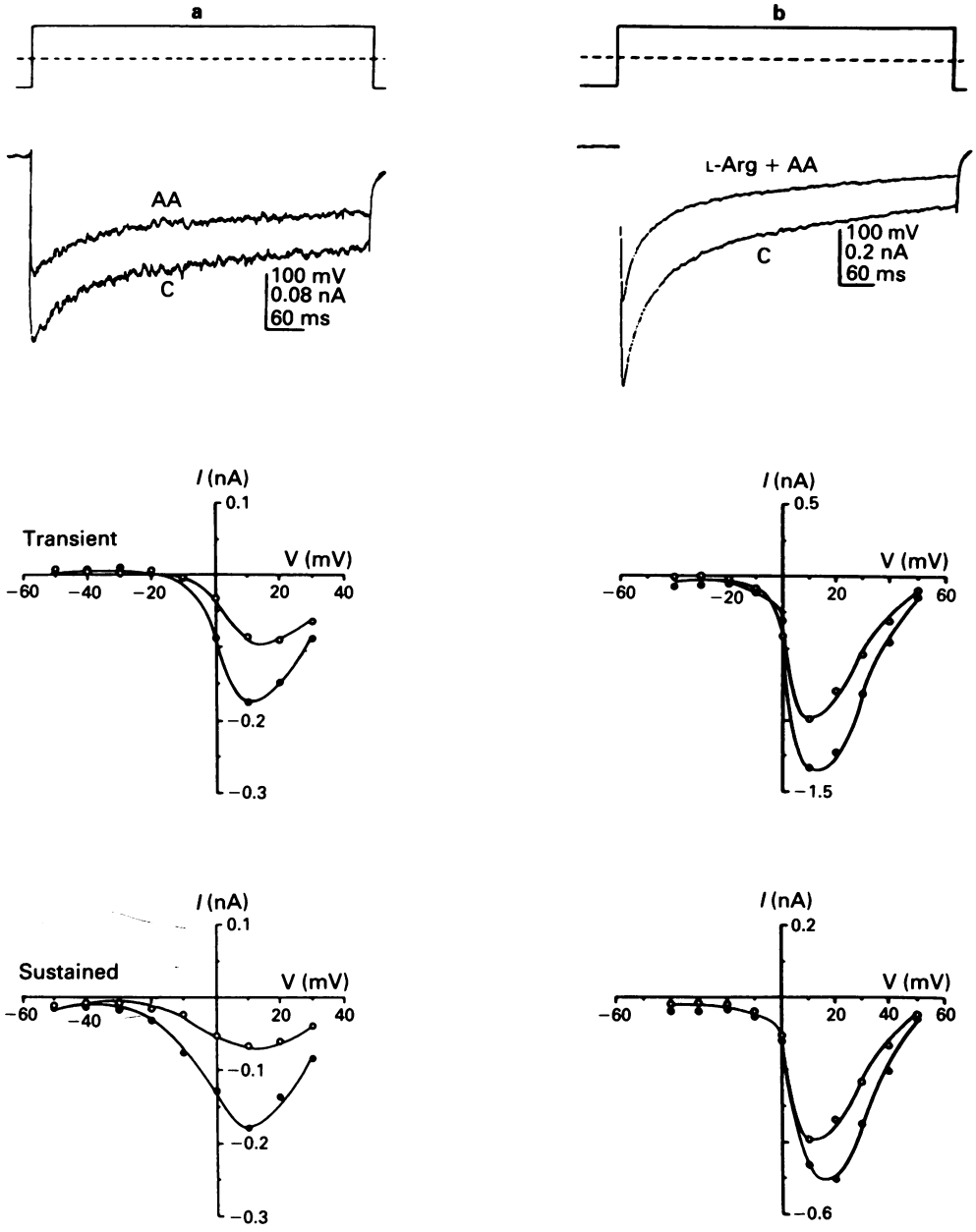

Figure 2 The action of arachidonic acid alone and arachidonic acid and L-arginine together on whole-cell calcium current. The upper panels give the effects of the drugs on the currents generated at + 10 mV from a holding potential of − 100 mV; the broken line indicates the level of − 50 mV. The middle and lower panels give the effects of the drugs on the transient and sustained currents respectively both before (●) and after (○) their puffer application; the voltages are the test potentials. (a) Effect of arachidonic (50 μM) on whole-cell transient and sustained calcium currents; the upper panel shows the currents evoked both before (C) and after (AA) exposure to arachidonic acid. (b) Effect of L-arginine (270 μM) and arachidonic acid (50 μM) on whole-cell transient and sustained calcium currents; in the upper panel are shown the currents evoked both before (C) and after (L-Arg + AA) exposure to the combined puff of L-arginine and arachidonic acid.

L-arginine were occluded by the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methylester (L-NAME; Figure 1c). After bathing the ganglion cells for at least 5 min in L-NAME (100 μM), a puff of L-arginine (270 μM) produced only a marginal decrease in the transient current, shifting it from -352 ± 57 pA to -335 ± 51 pA ($4 \pm 2\%$; $n = 6$; $P < 0.05$) during depolarization to +20 mV from a V_h of −100 mV. Under the same conditions, the sustained I_{Ca} was also only reduced by L-arginine to a small extent, namely from -235 ± 32 pA to -219 ± 28 pA ($7 \pm 2\%$; $n = 6$; $P < 0.05$). L-NAME clearly prevented the large depression in I_{Ca} due to L-arginine over the entire range of test potentials from −20 mV to +50 mV. These results indicate that a nitric oxide synthase system using L-arginine as a substrate can control both the transient and sustained components of high-voltage activated calcium current in parasympathetic ciliary neurones.

Arachidonic acid

Arachidonic acid also has an effect in reducing the whole-cell I_{Ca} in ciliary neurones (Figure 2a). During depolarization to +10 mV from a V_h of −100 mV, a puff of arachidonic acid (50 μM) reduced the transient I_{Ca} from -212 ± 40 pA to -153 ± 31 pA ($28 \pm 5\%$; $n = 13$; $P < 0.002$). Under the same conditions, arachidonic acid (50 μM) decreased the sustained I_{Ca} from -218 ± 43 pA to -162 ± 37 pA ($33 \pm 4\%$; $n = 13$; $P < 0.001$). The reductions in I_{Ca} occurred over the test potential range from −20 mV to +30 mV.

To exclude possible non-specific membrane effects of arachidonic acid, another fatty acid, oleic acid, was used as a control. During depolarization to +10 mV from a V_h of −100 mV, a puff of oleic acid (50 μM) reduced the transient I_{Ca} from -263 ± 11 pA to -255 ± 10 pA ($4 \pm 1\%$; $n = 6$; $P < 0.05$) and the sustained from -170 ± 7 pA to -165 ± 8 pA ($4 \pm 1\%$; $n = 6$; $P < 0.1$). These reductions in I_{Ca} occurred over the test potential range from −30 mV to +30 mV.

Possible interaction between NO and arachidonic acid

It has recently been shown that the cyclo-oxygenase-mediated prostaglandin metabolites of arachidonic acid can interact with the L-arginine:NO pathway via the activation of nitric oxide synthase (Gray *et al.*, 1991). It was therefore of interest to see whether or not the effects of arachidonic acid and L-arginine were additive. It was found that puffing arachidonic acid (50 μM) together with L-arginine (270 μM) onto ciliary neurones had the same effect on the sustained current as puffing arachidonic acid alone (Figure 2b): the sustained I_{Ca} was reduced from -346 ± 34 pA to -251 ± 32 pA ($29 \pm 3\%$; $n = 15$; $P < 0.001$); this is compared with a reduction of $33 \pm 4\%$ caused by a puff of arachidonic acid on its own. The transient I_{Ca} was reduced from -369 ± 44 pA to -273 ± 29 pA ($20 \pm 4\%$, $n = 15$; $P < 0.001$). These effects occurred over a test potential range from 0 mV to +50 mV, and suggest that arachidonic acid, or one of its metabolites, and L-arginine may be acting through a final common pathway to modulate I_{Ca}. To investigate this possibility further, indomethacin (20 μM), a specific inhibitor of cyclo-oxygenase, was introduced into the bathing solution prior to the puffing of arachidonic acid. Under these conditions, during depolarization to +20 mV from a V_h of −100 mV, a puff of arachidonic acid (50 μM) still caused a significant reduction in both the transient and sustained components of ciliary neurone I_{Ca} (Figure 3a): the transient I_{Ca} was reduced from -367 ± 88 pA to -253 ± 50 pA, a reduction of $28 \pm 2\%$ ($n = 7$; $P < 0.01$); the sustained was reduced from -414 ± 96 pA to -277 ± 49 pA, a reduction of $31 \pm 3\%$ ($n = 7$; $P < 0.02$). Similarly, the presence in the bathing solution of the specific nitric oxide synthase inhibitor, L-NAME (100 μM), also had no effect upon the depression of I_{Ca} caused by arachidonic acid (Figure 3b). Under these conditions, a puff of arachidonic acid reduced the transient I_{Ca} by $32 \pm 4\%$, shifting it from -237 ± 36 pA to -162 ± 25 pA ($n = 5$, $P < 0.001$), and reduced the sustained by $35 \pm 3\%$, decreasing it from -428 ± 55 pA to -275 ± 29 pA ($n = 5$; $P < 0.001$), effects which occurred during depolarizations to +10 mV from a V_h of −100 mV, and over the range of test potentials from 0 mV to +40 mV. Although the results indicate that in ciliary neurones arachidonic acid or one of its cyclo-oxygenase metabolites does not regulate nitric oxide synthase, the question as to whether the modulatory effects of arachidonic acid on calcium channels might be exerted through one of its leukotriene metabolites remained to be addressed. Using L-663,536 (MK-886), an inhibitor of leukotriene biosynthesis, the effects of arachidonic acid were then investigated (Figure 3c). With L-663,536 (3 μM) present in the bathing solution, a puff of arachidonic acid (50 μM) produced no significant change in the transient current, shifting it from -290 ± 13 pA to -291 ± 14 pA ($n = 6$; $P > 0.25$) during depolarization to +20 mV from a V_h of −100 mV. Under the same conditions, the sustained I_{Ca} was also only reduced by arachidonic acid to a small extent, namely from -323 ± 25 pA to -302 ± 24 pA ($6 \pm 2\%$; $n = 6$; $P < 0.05$). These results suggest that in ciliary neurones, the depression of whole-cell calcium current caused by arachidonic acid is in fact mediated by one of its leukotriene metabolites.

Discussion

The NO synthase present in avian ciliary neurones (Scott *et al.*, 1992) has been shown to be enzymatically active in the present work, converting L-arginine to NO (Garthwaite, 1991; for a review of the pathway see Moncada *et al.*, 1991) which then acts to modulate calcium currents. This enzyme has been shown to be active in all parts of the nervous system in which it has been found (Bredt & Snyder, 1991), whether by using antibodies to the synthase (Bredt *et al.*, 1990; Young *et al.*, 1992), or through localization of the mRNA for the enzyme (Bredt *et al.*, 1991), or via localization of NADPH diaphorase which is now known to be nitric oxide synthase (Dawson *et al.*, 1991; Hope *et al.*, 1991). Thus, the enteric inhibitory neurones to the gastrointestinal tract (Bennett *et al.*, 1963), which release neither noradrenaline nor acetylcholine (Bennett *et al.*, 1966), are now known to contain NO synthase and to release NO (see for example Li & Rand, 1990; Bult *et al.*, 1990; for a review see Rand, 1992). Likewise, the granule cells of the hippocampus are known to contain NO synthase (Bredt *et al.*, 1990) and here there is evidence that transmitter secretion from the perforant pathway is enhanced by the release of NO from the granule cells after activation of NMDA receptors on these cells (Errington *et al.*, 1991).

The action of NO on ciliary neurone calcium currents was to depress these uniformly, as it depresses the high-voltage activated calcium currents of the hippocampal neurones (Doerner & Alger, 1988). If similar effects occurred on presynaptic calcium currents, then a depression in transmitter secretion would be expected. However, as sodium nitroprusside is known to potentiate transmitter secretion in parasympathetic ganglia (Scott *et al.*, 1992) as well as in rat sympathetic ganglia (Briggs, 1992), this apparent dichotomy may be due to differences in the properties of postsynaptic calcium channels as compared with the presynaptic channels that mediate transmitter release. These calcium channels, which display transient calcium currents with a voltage-dependent inactivation different from that observed in the present work, have been identified at the preganglionic terminals of avian ciliary ganglia (Brosius *et al.*, 1990; Stanley & Goping, 1991; Stanley, 1992). It will therefore be necessary to examine the effects of NO on these presynaptic channels in order to determine whether NO is likely to enhance transmitter release by acting differentially on calcium channels present in nerve terminals.

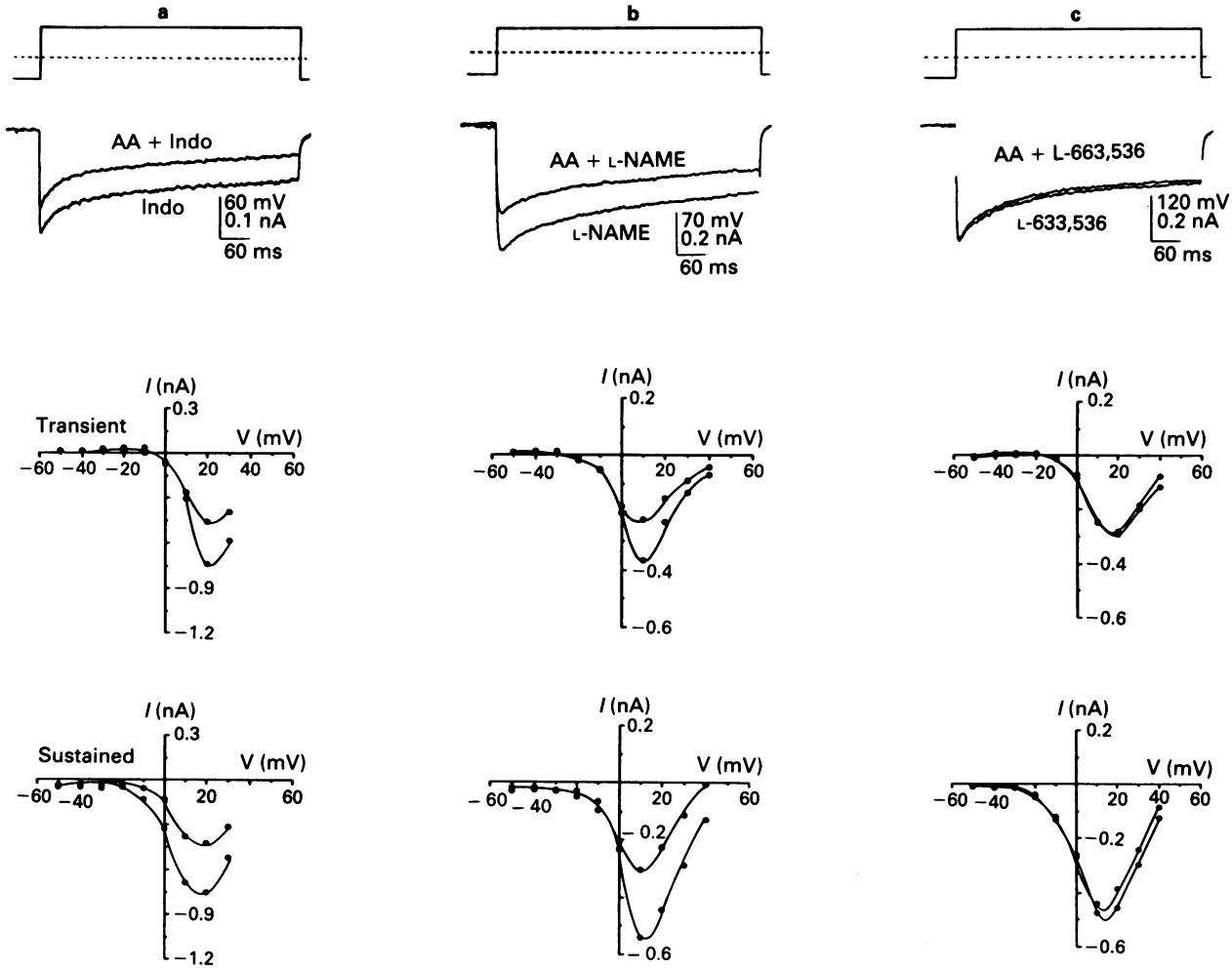

Figure 3 The effects of arachidonic acid on whole-cell calcium current in the presence of indomethacin, L-N^G-nitroarginine methylester (L-NAME) and L-663,536. The upper panels in (a) and (c) give the effects on the currents generated at + 20 mV from a holding potential (V_h) of − 100 mV; in (b) is shown the effect on the currents generated at + 10 mV from a V_h of − 100 mV; the broken line in each case indicates the level of − 50 mV. The middle and lower panels give the effects of arachidonic acid on the transient and sustained currents respectively both before (●) and after (○) its puffer application; the voltages are the test potentials. (a) Effect of arachidonic acid (50 μM) on whole-cell transient and sustained calcium currents in the presence of indomethacin (20 μM) in the bathing solution; the upper panel shows the currents evoked in the presence of indomethacin alone (Indo) and after exposure to a puff of arachidonic acid (AA + Indo) given 10 s before the observation was made in the presence of indomethacin. (b) Effect of arachidonic acid (50 μM) on transient and sustained calcium currents in the presence of L-NAME (100 μM) in the bathing solution; the upper panel shows the currents evoked in the presence of L-NAME alone (L-NAME) and after exposure to a puff of arachidonic acid (AA + L-NAME) given 10 s before the observation was made in the presence of L-NAME. (c) Effect of arachidonic acid (50 μM) on transient and sustained calcium currents in the presence of L-663,536 (3 μM) in the bathing solution; the upper panel shows the currents evoked in the presence of L-663,536 alone (L-663,536) and after exposure to a puff of arachidonic acid (AA + L-663,536) given 10 s before the observation was made in the presence of L-663,536.

The effect of arachidonic acid on transmission in the ciliary ganglion has not been determined. Arachidonic acid increases the efficacy of transmission between the perforant pathway and granule cells in the hippocampus (Williams *et al.*, 1988) and this effect can be blocked by the phospholipase A_2 and lipoxygenase inhibitor, nordihydroguaiaretic acid (Lynch *et al.*, 1989). This suggests that endogenous arachidonic acid or one of its lipoxygenase metabolites is responsible for the increased efficacy of transmission, perhaps by facilitating the NO synthase system (O'Dell *et al.*, 1991). However arachidonic acid or one of its lipoxygenase metabolites depresses the high-voltage activated L-type calcium currents in hippocampal neurones (Keyser & Alger, 1990). In the present study, arachidonic acid also reduced the high-voltage activated calcium current, and by use of L-663,536 (MK-886; see Gillard *et al.*, 1989) this effect was shown to be mediated by its leukotriene metabolites. A depression of calcium current is not to be expected if the action of arachidonic acid is to enhance transmitter release by increasing the efficacy of presynaptic calcium channels. Again, it would seem that a direct analysis of the effects of arachidonic acid on presynaptic calcium channels is required.

Arachidonic acid is converted to prostaglandins by cyclooxygenase (for a review see Piomelli & Greengard, 1990), and these are known to potentiate or even initiate the events leading to activation of the L-arginine:NO pathway (Gray *et al.*, 1991). In the present study, the inhibition of calcium current caused by arachidonic acid was found not to be additive with that caused by nitric oxide, and this suggested that both may work through a final common pathway in ciliary neurones. However, as neither indomethacin nor L-NAME affected the depression of whole-cell calcium current caused by arachidonic acid, it seems likely that in these parasympathetic neurones, arachidonic acid and nitric oxide act via independent pathways to modulate high-voltage activated calcium channels.

References

BENNETT, M.R., BURNSTOCK, G. & HOLMAN, M.E. (1963). The effect of potassium and chloride ions on the inhibitory potential recorded in the guinea-pig taenia coli. *J. Physiol.*, **169**, 33P.

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 cultured avian ciliary ganglia. *Br. J. Pharmacol.*, **106**, 25–32.

BREDT, D.S., HWANG, P.M., GLATT, C.E., LOWENSTEIN, C., REED, R.R. & SNYDER, S.H. (1991). Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature*, **351**, 714–718.

BREDT, D.S., HWANG, P.M. & SNYDER, S.H. (1990). Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature*, **347**, 768–770.

BREDT, D.S. & SNYDER, S.H. (1992). Nitric oxide, a novel neuronal messenger. *Neuron*, **8**, 3–11.

BRIGGS, C.A. (1992). Potentiation of nicotinic transmission in the rat superior cervical sympathetic ganglion: effects of cyclic GMP and nitric oxide generators. *Brain Res.*, **573**, 139–146.

BROSIUS, D.C., HACKETT, J.T. & TUTTLE, J.B. (1990). Pre-synaptic calcium currents evoking quantal transmission from avian ciliary ganglion neurons. *Synapse*, **5**, 313–323.

BUG, W., ROLE, L., SIEGELBAUM, S.A. & SIMMONS, L. (1989). Arachidonic acid inhibits calcium current in chick sympathetic neurons. *Neurosci. Soc. Abstr.*, **15**, 177.

BULT, H., BOECKXSTAENS, G.E., PELCKMANS, P.A., JORDAENS, F.H. VAN MAERCKE, Y.M. & HERMAN, A.G. (1990). Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature*, **345**, 346–347.

CLAPP, L.H. & GURNEY, A.M. (1991). Modulation of calcium movements by nitroprusside in isolated vascular smooth muscles. *Eur. J. Physiol.*, **418**, 462–470.

DAWSON, T.M., BREDT, D.S., FOTUHI, M., HWANG, P.M. & SNYDER, S.H. (1991). Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 7797–7801.

DOERNER, D. & ALGER, B.E. (1988). Cyclic GMP depresses hippocampal Ca^{2+} current through a mechanism independent of cGMP-dependent protein-kinase. *Neuron*, **1**, 693–699.

ERRINGTON, M.L., LI, Y.G., MATTHIES, H., WILLIAMS, J.H. & BLISS, T.V.P. (1991). The nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine reduces the magnitude of long-term potentiation in the dentate gyrus but not in area CA1 of the hippocampus *in vitro*. *Neurosci. Soc. Abstr.*, **17**, 951.

FOX, A.P., NOWYCKY, M.C. & TSIEN, R.W. (1987a). Kinetic and pharmocological properties distinguishing three types of calcium current in chick sensory neurones. *J. Physiol.*, **394**, 149–172.

FOX, A.P., NOWYCKY, M.C. & TSIEN, R.W. (1987b). Single-channel recordings of three types of calcium channels in chick sensory neurones. *J. Physiol.*, **394**, 173–200.

GARTHWAITE, J. (1991). Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci.*, **14**, 60–67.

GILLARD, J., FORD-HUTCHINSON, A.W., CHAN, C., CHARLESON, S., DENIS, D., FOSTER, A., FORTIN, R., LEGER, S., MCFARLANE, C.S., MORTON, H., PIECHUTA, H., REINDEAU, D., ROUZER, C.A., ROKACH, J., YOUNG, R., MACINTYRE, D.E., PETERSON, L., BACH, T., EIERMANN, G., HOPPLE, S., HUMES, J., HUPE, L., LUELL, S., METZGER, J., MEURER, R., MILLER, D.K., OPAS, E. & PACHOLOK, S. (1989). L-663,536 (MK-886) (3-[1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.*, **67**, 456–464.

GRAY, G.A., SCHOTT, C., JULOU-SCHAEFFER, G., FLEMING, I., PARRATT, J.R. & STOCLET, J.C. (1991). The effect of inhibitors of the L-arginine-nitric oxide pathway on endotoxin induced loss of vascular responsiveness in anaesthetized rats. *Br. J. Pharmacol.*, **103**, 1210–1224.

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.

HOPE, B.T., MICHAEL, G.J., KNIGGE, K.M. & VINCENT, S.R. (1991). Neuronal NADPH diaphorase is a nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 2811–2814.

KEYSER, D.O. & ALGER, B.E. (1990). Arachidonic acid modulates hippocampal calcium current via protein kinase C and oxygen radicals. *Neuron*, **5**, 545–553.

LI, G.G. & RAND, M.J. (1990). Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic, non-cholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. *Eur. J. Pharmacol.*, **191**, 303–309.

LYNCH, M.A., ERRINGTON, M.L. & BLISS, T.V.P. (1989). Nordihydroguiaretic acid blocks the synaptic component of long-term potentiation and the associated increases in release of glutamate and arachidonate: an *in vivo* study in the dentate gyrus of the rat. *Neurosci.*, **30**, 693–701.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43**, 109–143.

O'DELL, T.J., HAWKINS, R.D., KANDEL, E.R. & ARANCIO, I. (1991). Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 11285–11289.

PIOMELLI, D. & GREENGARD, P. (1990). Lipoxygenase metabolites of arachidonic acid in neuronal transmembrane signalling. *Trends Pharmacol. Sci.*, **11**, 367–373.

PIOMELLI, D., VOLTERRA, A., DALE, N., SIEGELBAUM, S.A., KANDEL, E.R., SCHWARTZ, J.H. & BELARDETTI, F. (1987). Lipoxygenase metabolites of arachidonic acid as second messengers for pre-synaptic inhibition of *Aplysia* sensory cells. *Nature*, **328**, 38–43.

RAND, M.J. (1992). New perspectives in neurotransmission: nitric oxide, the mediator of nitergic transmission. *Proc. Aust. Physiol. Pharmacol. Soc.*, **23**, 1–16.

SCHROEDER, J.E., FISCHBACH, P.S., MAMO, M. & MCCLOSKEY, E.W. (1990). Two components of high-threshold Ca^{2+} current inactivate by different mechanisms. *Neuron*, **5**, 445–452.

SCOTT, T.R.D., NICHOL, K., 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.

SCOTT, R.H., PEARSON, H.A. & DOLPHIN, A.C. (1991). Aspects of vertebrate neuronal voltage-activated calcium currents and their regulation. *Prog. Neurobiol.*, **36**, 485–520.

SHER, E. & CLEMENTI, F. (1991). ω-conotoxin-sensitive voltage-operated calcium channels in vertebrate cells. *Neurosci.*, **42**, 301–307.

STANLEY, E.F. (1992). Single calcium channels in a cholinergic presynaptic nerve terminal. *Neuron*, **7**, 585–591.

STANLEY, E.F. & GOPING, G. (1991). Characterization of a calcium current in a vertebrate cholinergic nerve terminal. *J. Neurosci.*, **11**, 985–993.

SWANDULLA, D., CARBONE, E. & LUX, H.D. (1991). Do calcium channel classifications account for neuronal calcium diversity? *Trends Neurosci.*, **14**, 46–51.

TARE, M., PARKINGTON, H.C., COLEMAN, H.A., NEILD, T.O. & DUSTING, G.J. (1990). Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. *Nature*, **346**, 69–71.

WILLIAMS, J.H., ERRINGTON, M.L., LYNCH, M.A. & BLISS, T.V.P. (1988). Arachidonic acid induces long-term activity-dependent enhancement of synaptic transmission in the hippocampus. *Nature*, **341**, 739–742.

YOUNG, H.M., FURNESS, J.B., SHUTTLEWORTH, C.W.R., BREDT, D.S. & SNYDER, S.H. (1992). Co-localization of nitric oxide synthase immunoreactivity and NADPH diaphorase staining in neurones of the guinea-pig intestine. *Proc. Aust. Physiol. Pharmacol Soc.*, **23**, 45P.

(Received July 7, 1992
Revised January 4, 1993
Accepted February 8, 1993)