British Journal of Pharmacology (1996) 119, 527-532

The nitric oxide-cyclic GMP pathway and synaptic plasticity in the rat superior cervical ganglion

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1 We have investigated the possibility that nitric oxide (NO) and soluble guanylyl cyclase, an enzyme that synthesizes guanosine 3':5'-cyclic monophosphate (cyclic GMP) in response to NO, contributes to plasticity of synaptic transmission in the rat isolated superior cervical ganglion (SCG).

2 Exposure of ganglia to the NO donor, nitroprusside, caused a concentration-dependent accumulation of cyclic GMP which was augmented in the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine. The compound, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a selective inhibitor of soluble guanylyl cyclase, completely blocked this cyclic GMP response.

3 As assessed by extracellular recording, nitroprusside (100 μM) and another NO donor, S-nitrosoglutathione (30 μM) increased the efficacy of ganglionic synaptic transmission in response to electrical stimulation of the preganglionic nerve, an effect that was reversible and which could be replicated by the cyclic GMP analogue, 8-bromo-cyclic GMP. Ganglionic depolarizations resulting from stimulation of nicotinic receptors with carbachol were not increased by nitroprusside. The potentiating actions of the NO donors on synaptic transmission, but not that of 8-bromo-cyclic GMP, were inhibited by ODQ.

4 Brief tetanic stimulation of the preganglionic nerve resulted in a long-term potentiation (LTP) of synaptic transmission that was unaffected by ODQ, either in the absence or presence of the NO synthase inhibitor, N^G-nitro-L-arginine (L-NOARG, 100 μM). A lack of influence of L-NOARG was confirmed in intracellular recordings of LTP of the excitatory postsynaptic potential. Furthermore, under conditions where tetanically-induced LTP was saturated, nitroprusside was still able to potentiate synaptic transmission, as judged from extracellular recording.

5 We conclude that NO is capable of potentiating ganglionic neurotransmission and this effect is mediated through the stimulation of soluble guanylyl cyclase and the accumulation of cyclic GMP. However, this potentiation is distinct from LTP of nicotinic synaptic transmission, in which neither NO nor soluble guanylyl cyclase appear to participate.

Keywords: Nitric oxide; cyclic GMP; sympathetic ganglia; long-term potentiation; ODQ; soluble guanylyl cyclase

Introduction

Long-term potentiation (LTP) of synaptic transmission is a well-known example of synaptic plasticity that has been most extensively studied at glutamatergic synapses in the hippocampus. A number of second messenger systems have been implicated in LTP in this region, among them the freely diffusible molecule, nitric oxide (NO) (Böhme *et al.*, 1991; O'Dell *et al.*, 1991; Schuman & Madison, 1991). An established action of NO is to stimulate the soluble form of the enzyme, guanylyl cyclase, leading to the cellular accumulation of guanosine 3':5'-cyclic monophosphate (cyclic GMP), although several other transduction pathways have also been proposed (Garthwaite & Boulton, 1995). The recent identification of a selective inhibitor of soluble guanylyl cyclase, the quinoxaline derivative 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (Garthwaite *et al.*, 1995) has allowed the role of this enzyme in NO signal transduction to be tested. Using ODQ we have shown that soluble guanylyl cyclase mediates the action NO in hippocampal LTP (Boulton *et al.*, 1995). In this instance, the resultant generation of cyclic GMP probably functions by activating a cyclic GMP-dependent protein kinase (Zhuo *et al.*, 1994).

LTP can also be observed in the peripheral nervous system, most notably in sympathetic ganglia (Brown & McAfee, 1982; Briggs *et al.*, 1985a; Briggs & McAfee, 1988). In this tissue, LTP is induced by brief tetanic stimulation of the preganglionic nerve and is believed to be expressed, at least in part, presynaptically as it is accompanied by enhanced release of acetylcholine (Briggs *et al.*, 1985b) but the underlying mechanisms remain to be elucidated. A number of observations provide circumstantial evidence that the NO-cyclic GMP pathway could participate. Immunocytochemistry and histochemistry have demonstrated that NO synthase is contained within preganglionic nerve fibres (Dun *et al.*, 1993; Morris *et al.*, 1993; Okamura *et al.*, 1995) and tetanic stimulation of the preganglionic nerve is likely to result in NO formation since this treatment causes an increased cyclic GMP content of rat SCG which can be blocked by inhibition of NO synthase (Sheng *et al.*, 1992). Moreover, it has been shown that ganglionic neurotransmission can be enhanced by NO donating compounds (Briggs, 1992).

The aim of the present experiments was to determine if the potentiation of synaptic transmission brought about by NO is mediated through soluble guanylyl cyclase and to test the possibility that the NO-cyclic GMP pathway contributes to tetanically-induced LTP.

Methods

Tissue preparation

Superior cervical ganglia together with their preganglionic and postganglionic nerve trunks (tied at each end with suture

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thread) were dissected from young adult (5–8 weeks) Wistar rats, of either sex, under urethane anaesthesia (Marsh, 1989). For biochemical studies and for extracellular recording, the ganglia were fully desheathed whereas, for the purpose of intracellular recording, the sheath was split longitudinally so as to expose ganglion neurones. The ganglia were kept until needed at room temperature in a Krebs solution containing (mM): NaCl 120, KCl 5.6, $CaCl_2$ 2, $NaHCO_3$ 26, $MgSO_4$ 1.19, KH_2PO_4 1.18, and glucose 11, equilibrated with 5% CO_2 in O_2.

Cyclic GMP measurement

Ganglia were incubated for 1–1.5 h in the Krebs solution at 30±1°C in a shaking water bath before the addition of test compounds. Preincubation periods with the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 1 mM) or with ODQ (10 μM) were 1 h and 15 min respectively. The tissues were exposed to different concentrations of nitroprusside for 5 min, inactivated in boiling hypotonic Tris-EDTA buffer, and their protein and cyclic GMP contents measured as described previously (Garthwaite & Garthwaite, 1987).

Extracellular grease-gap recording

A ganglion was installed in a three-chambered bath so that the body of the ganglion was positioned in the central compartment which was perfused with gassed Krebs solution (30±1°C) at a rate of 2 ml min^{-1}. The preganglionic cervical sympathetic and postganglionic internal carotid nerves projected through high resistance grease barriers into the side compartments. Differential recordings of the ganglionic response to supramaximal (20% above maximal) square wave stimuli (0.1 ms duration) delivered to the preganglionic nerve at 0.02 Hz, were made with $Ag/AgCl_2$ electrodes (Marsh, 1989). The muscarinic component of synaptic transmission was blocked by atropine (2 μM) and all solutions contained the nicotinic antagonist, hexamethonium, at a concentration (300 μM) that reduced the amplitude of the compound action potential (CAP) by about 60% (Briggs *et al.*, 1985a). Potentiations caused by NO-releasing compounds were calculated as the peak CAP amplitude relative to a 30 min average baseline. The d.c. potential changes occurring in response to exogenous agonist application were displayed on a chart recorder. Carbachol (100 μM) was tested in the presence of atropine at a concentration (2 μM) sufficient to negate the muscarinic component of its action (D.A. Brown, personal communication).

For investigating LTP, baseline (0.02 Hz) recordings of the CAP amplitude were first made over 30 min and then a tetanus comprising 75 stimuli at a frequency of 5 Hz was delivered to the preganglionic nerve trunk (Briggs *et al.*, 1985a). Baseline (0.02 Hz) stimulation was then resumed. The resulting changes in CAP amplitude were expressed as a fraction of the mean control amplitude measured during the 30 min period preceding the tetanus.

Intracellular recording

The isolated SCG was secured to a Sylgard-coated recording chamber by stainless steel entomological pins (size A1), positioned through the suture knots around the nerve trunks and the ganglion sheath. The ganglion was superfused (1 ml min^{-1}) with gassed Krebs solution at 30±1°C. Intracellular recordings were made of the excitatory postsynaptic potential (e.p.s.p.) in ganglion neurones, held at −60 mV, using sharp electrodes filled with 3 M KCl (about 70 M Ω resistance). The muscarinic slow e.p.s.p. was blocked by atropine (2 μM) and action potential firing prevented by hexamethonium (300 – 600 μM). Input resistance, measured by injecting a 50 ms, 0.1 nA hyperpolarizing current pulse, was monitored throughout the recording period. Baseline e.p.s.ps were recorded (0.05 Hz stimulation of the preganglionic nerve with 0.1 ms square wave pulses at 20% above threshold voltage) for at least 15 min before LTP was elicited by tetanic stimulation (20 Hz for 20 s at 3-times threshold voltage) of the preganglionic trunk. E.p.s.ps were quantified by measurement of their peak height.

Statistics

Data are expressed as mean±s.e.mean of n values. All statistical comparisons were made using Student's t test and $P<0.05$ considered significant.

Materials

ODQ was a gift of Dr T. Honoré (NovoNordisk, Denmark) and S-nitrosoglutathione was synthesized at the Wellcome Research Laboratories. Sodium nitroprusside, 3-isobutyl-1-methylxanthine, 8-bromo-cyclic GMP, and N^G-nitro-L-arginine were all obtained from Sigma. Solutions of the NO-donors were prepared immediately before use.

Results

Inhibition of NO-induced cyclic GMP accumulation by ODQ

Resting cyclic GMP levels in the ganglia were around 1 pmol mg^{-1}protein. Sodium nitroprusside (SNP), a compound which spontaneously releases NO, caused a progressive increase in cyclic GMP levels when added at concentrations of 10 μM to 1 mM (Figure 1a). The cyclic GMP response to 100 μM nitroprusside was amplified 30 fold in the presence of the broad-spectrum phosphodiesterase inhibitor, IBMX (1 mM) (Figure 1b). In the presence of ODQ (10 μM), the nitroprusside-induced accumulation of cyclic GMP was abolished (Figure 1b).

Enhancement of synaptic transmission through the NO-cyclic GMP pathway

Confirming the work of Briggs (1992), NO donor compounds were found to increase the amplitude of the extracellularly-recorded compound action potential (CAP) elicited by low frequency (0.02 Hz) stimulation of the preganglionic nerve (Figure 2a, c). This effect was relatively slow to develop, peaking about 15 min after initial (10 min) application, and was completely, albeit slowly, reversible. The mean relative increases in CAP amplitudes caused by nitroprusside (100 μM) and S-nitrosoglutathione (SNOG; 30 μM) were similar at 27±3% ($n=20$) and 23±2%, ($n=6$), respectively. ODQ (30 min preincubation) inhibited the NO donor-induced increase in CAP amplitude (Figure 2a, c) in a concentration-dependent manner ($IC_{50}\approx3$ μM verses 100 μM nitroprusside, Figure 2b). The cyclic GMP analogue, 8-bromo-cyclic GMP (30 μM, 10 min), also produced a slowly developing potentiation of CAP amplitude (34±8%, $n=4$) but this effect was not blocked by ODQ (Figure 3a, b).

To check if the augmentation of neurotransmission brought about by NO might be effected postsynaptically, the effect of nitroprusside on carbachol-induced depolarizations were investigated under conditions where muscarinic receptors were blocked by 2 μM atropine. A 75 s exposure to 100 μM carbachol resulted in a depolarizing d.c. shift of 0.60±0.12 mV ($n=3$). After 10 min exposure to 1 mM nitroprusside, when the simultaneously-recorded CAP amplitude was enhanced by 36±8%, carbachol-induced depolarizations were slightly reduced to 88±3% of controls ($n=3$, $P<0.05$) (Figure 4). Nitroprusside (1 mM) itself caused a small depolarization (peaking at 0.17±0.07 mV after 90 s, $n=3$).

The NO-cyclic GMP pathway and LTP

A 15 s, 5 Hz tetanic stimulus delivered to the preganglionic nerve resulted in a LTP of the CAP amplitude which lasted for

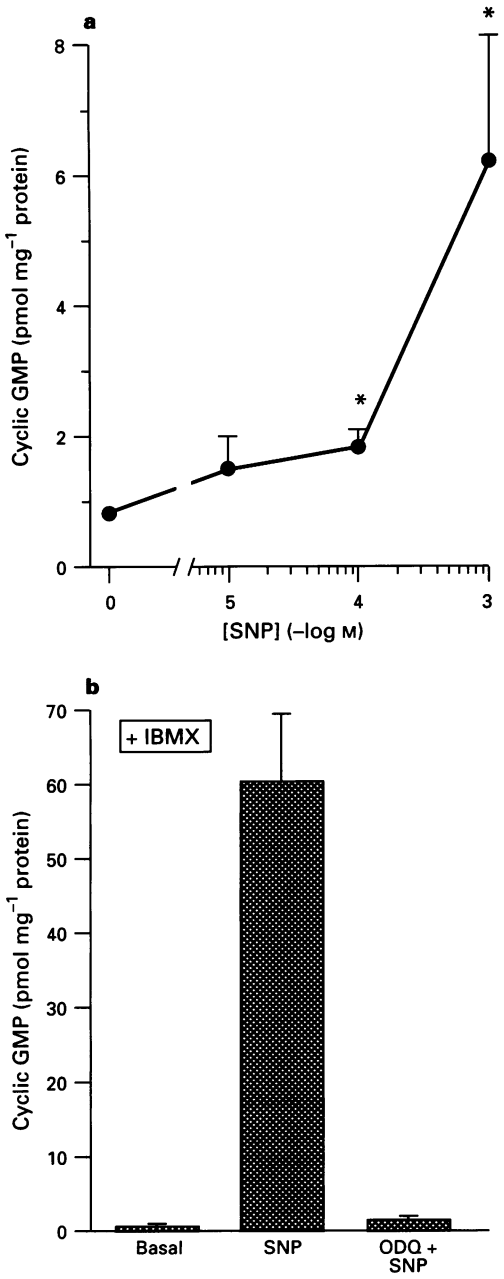

Figure 1 Nitroprusside-induced accumulation of cyclic GMP is blocked by ODQ in the rat SCG. (a) Concentration-response curve for elevation in ganglionic cyclic GMP levels by nitroprusside (SNP) (*$P<0.05$). (b) Effect of 10 μM ODQ on cyclic GMP accumulation in response to 100 μM nitroprusside in the presence of 1 mM IBMX. Each data point or column represents the mean ± s.e.mean from 3 to 4 ganglia.

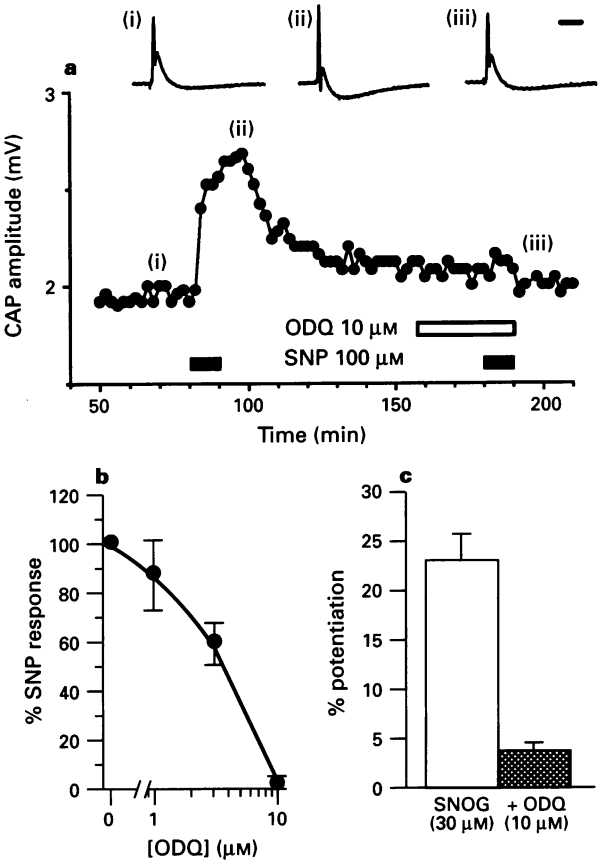

Figure 2 NO donors enhance ganglionic transmission through stimulation of guanylyl cyclase. (a) A single experiment, showing that nitroprusside (SNP, solid horizontal bar) potentiates neurotransmission, measured as the CAP amplitude (mV), in a manner that is blocked by 10 μM ODQ (open horizontal bar). The sample traces were taken at the time points indicated by (i), (ii) and (iii); scale bar = 50 ms. (b) Concentration-dependence of the inhibition of nitroprusside-induced potention (100 μM) by ODQ. Each point is the mean ± s.e.mean for at least 3 ganglia. (c) Potentiation of transmission by 30 μM S-nitrosoglutathione (SNOG) in the absence and presence of 10 μM ODQ (means ± s.e.mean; $n=5$).

the remainder of the recordings, that is, for at least 1 h (Figure 5a, b). In initial experiments, ganglia were exposed to 10 μM ODQ for 30 min prior to and during the tetanus and then ODQ-free Krebs solution was reinstated. An hour after the tetanus, the potentiation of CAP amplitude in control and ODQ-treated ganglia did not differ significantly (27 ± 2%, $n=5$ and 22 ± 3%, $n=4$, respectively) (Figure 5a). Addition of the NO synthase inhibitor, N^G-nitro-L-arginine (L-NOARG) (100 μM), together with ODQ and extending the exposure to straddle the tetanus by 1 h on each side, proved to be similarly ineffective: the mean enhancement of CAP amplitude 60 min after the tetanus was 22% ± 4% in control ganglia ($n=7$) and 19 ± 8% ($n=5$) in treated ganglia (Figure 5b). Neither inhibitor had a significant effect on baseline synaptic transmission.

The validity of these results was checked by intracellular recording of nicotinic e.p.s.ps. Tetanic stimulation (20 Hz, 20 s) of the preganglionic nerve resulted in LTP of the e.p.s.p. amplitude amounting to a 31 ± 1%, ($n=5$) increase over baseline values after 30 min. L-NOARG (100 μM), applied from 20 min before the tetanus until the end of the experiment, had no effect on baseline e.p.s.p. amplitude, nor did it influence the degree of LTP. The mean potentiation 30 min after tetanus in the presence of L-NOARG was 29 ± 1% ($n=5$) (Figure 5c).

Effect of NO when LTP is saturated

Repeated (up to 6) tetanic stimuli (5 Hz, 15 s) applied to the preganglionic nerve at 30 min intervals resulted in a saturation of LTP measured extracellularly as the CAP amplitude. Subsequent tetanic stimulation produced only a short-term potentiation (c.f. Brown & McAfee, 1982). As assessed 30 min after the final tetanus, the maximum LTP was 52% ± 9% ($n=6$) above baseline (Figure 6a). Comparisons were made of the effect of perfusing nitroprusside (10 min, 100 μM) before and after this treatment. In both cases, synaptic transmission was potentiated, the values (17 ± 1% before and 19 ± 2% afterwards; $n=6$) (Figure 6a, b) not being significantly different.

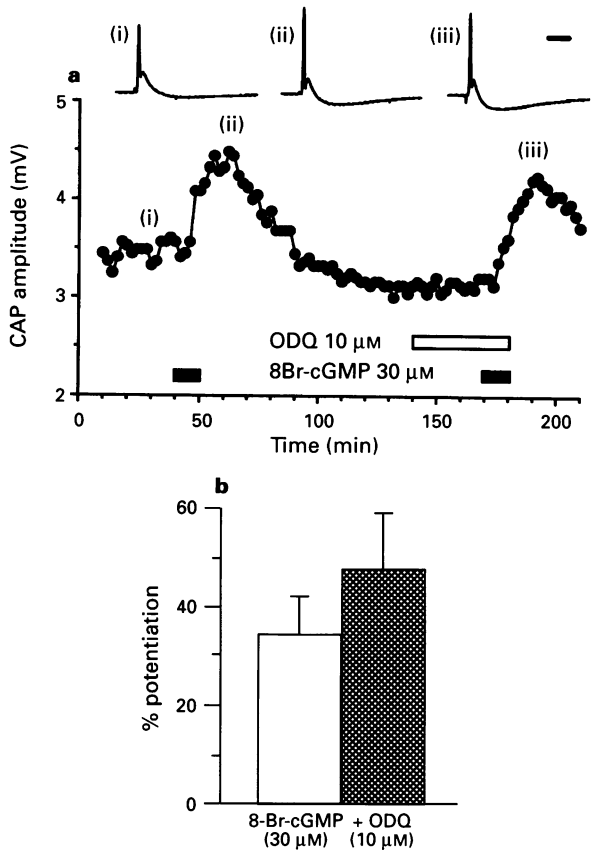

Figure 3 Potentiation of ganglionic transmission by 8-bromo-cyclic GMP (8Br-cGMP) is resistant to ODQ. (a) A single representative experiment showing reversible increase in CAP amplitude in response to 8-bromo-cGMP (solid horizontal bars) in the absence and presence of 10 μM ODQ (open horizontal bar). The sample traces were taken at the times indicated by (i), (ii) and (iii); scale bar = 50 ms. (b) Pooled data (means ± s.e.mean) from 4 ganglia.

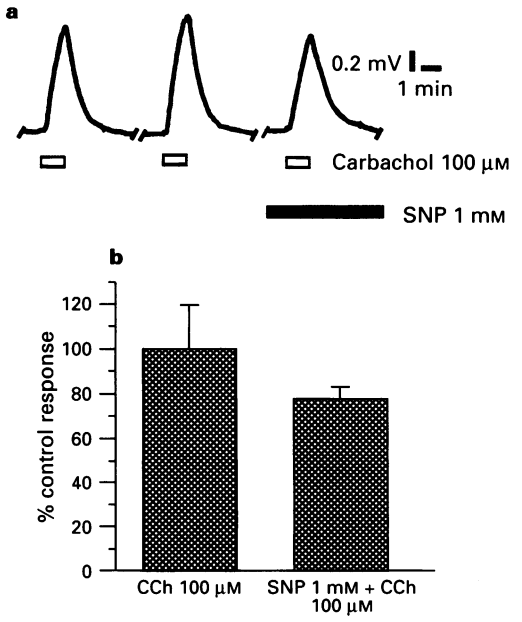

Figure 4 Effect of nitroprusside on ganglion depolarizations elicited by the cholinoceptor agonist, carbachol (CCh, 100 μM). (a) Sample traces of the d.c. potential changes in response to perfusion of carbachol (open horizontal bar) before and during application of nitroprusside (SNP, 1 mM, solid horizontal bar). (b) Pooled results expressed as percentage of control depolarization amplitude (means ± s.e.mean; $n = 3$).

Discussion

The NO cyclic GMP pathway and synaptic transmission in the rat SCG

At one time, the SCG was a popular model tissue for trying to understand the role of cyclic nucleotides in synaptic transmission, although no clear picture emerged (Busis *et al.*, 1978). The identification of NO as a neural messenger molecule capable of eliciting cyclic GMP accumulation by activating soluble guanylyl cyclase (Garthwaite & Boulton, 1995) has added a previously missing element. Immunocytochemistry and histochemistry have shown that the preganglionic nerves represent the main site of NO synthesis in the SCG (Dun *et al.*, 1993; Morris *et al.*, 1993; Okamura *et al.*, 1995) and evidence exists that invasion of the nerves by action potentials stimulates NO formation Ca^{2+}-dependently (Sheng *et al.*, 1992).

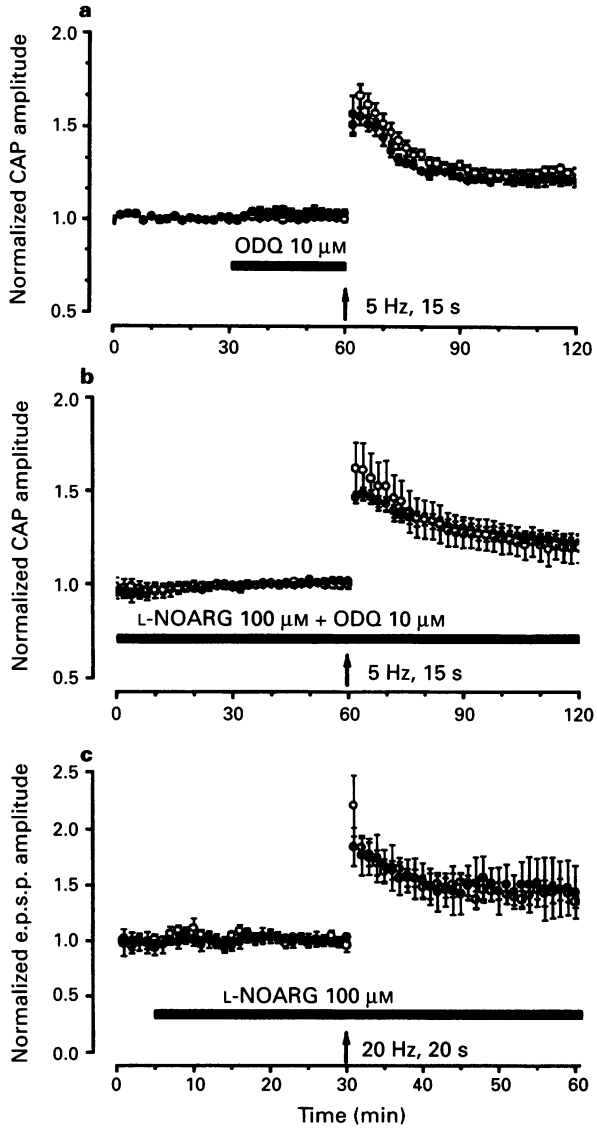

Figure 5 Effect of inhibitors of NO synthase and soluble guanylyl cyclase on tetanically induced LTP. (a) Normalized CAP amplitude in the absence (○) or presence (●) of 10 μM ODQ. (b) Normalized CAP amplitude in the absence (○) or presence (●) of 100 μM L-NOARG plus 10 μM ODQ. (c) Normalized e.p.s.p. amplitude measured by intracellular recording in the absence (○) or presence (●) of 100 μM L-NOARG. Horizontal solid bars indicate times of drug applications; arrows show when tetanic stimuli were delivered. The data are means ± s.e.mean from 4–5 ganglia and the data have been normalized to the mean amplitudes during the 30 min period immediately preceding the tetanus.

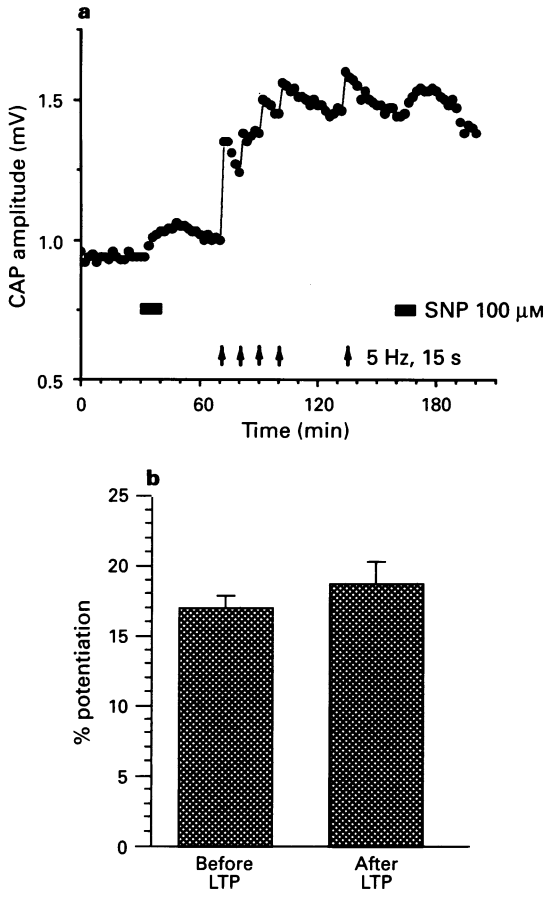

Figure 6 Effect of saturation of LTP on nitroprusside-induced potentiation of synaptic transmission. (a) Representative experiment showing the enhancement of CAP amplitude by 100 μM nitroprusside (SNP, solid horizontal bars) before and after LTP was saturated by repeated tetanic stimuli (5 Hz, 15 s; arrows). (b) Pooled data showing the mean potentiations ($\pm$s.e.mean) caused by nitroprusside in 6 ganglia.

Using the new inhibitor, ODQ, we have shown that, as in brain slices and endothelial cells exposed to NO donors (Garthwaite *et al.*, 1995), nitroprusside increases ganglionic cyclic GMP levels through activation of soluble guanylyl cyclase, presumably with NO as the intermediate. In addition, two structurally-distinct NO donors caused an augmentation of ganglionic synaptic transmission, a finding that is consistent with previous results from both mammalian (Briggs, 1992) and avian (Scott & Bennett, 1993) autonomic ganglia. Since this effect was blocked by ODQ and could be mimicked by cyclic GMP analogues (see also Briggs, 1992), it can be concluded that it is mediated by soluble guanylyl cyclase.

The anatomical targets generating cyclic GMP in response to NO in this tissue are not clearly identified at present. Haemoglobin, which scavenges NO, reduces the cyclic GMP response to electrical stimulation (Sheng *et al.*, 1992), suggesting that NO acts intercellularly to produce this response. Cyclic GMP immunocytochemistry is reported to label postsynaptic cells after ganglia are stimulated with high K^+ concentrations or carbachol (De Vente *et al.*, 1987). However, the increase in cyclic GMP levels occurring in response to sodium azide (which can be degraded to yield NO) is not reduced by axotomy or denervation of ganglia, suggesting that non-neuronal cells may also participate significantly (Ando *et al.*, 1983).

In the present experiments, ganglionic depolarizations caused by activation of nicotinic receptors with carbachol were not increased by a concentration of nitroprusside that enhanced synaptic transmission by 36%, arguing against a postsynaptic site of action. This result concurs with findings from chick ciliary ganglia in which nitroprusside and 8-bromo-cyclic GMP appeared to act presynaptically to enhance the amplitude of the e.p.s.p. (Lin & Bennett, 1994).

The NO-cyclic GMP pathway and tetanically-induced LTP

Given evidence for the presence of the NO-cyclic GMP pathway in the SCG, that stimulation of this pathway is associated with a potentiation of synaptic transmission, and that the pathway is activated by the type of preganglionic nerve stimulation that results in LTP, the idea that NO-cyclic GMP signalling participated in LTP was an attractive one, particularly in the light of recent results implicating this mechanism in LTP in the hippocampus (Boulton *et al.*, 1995). Nevertheless, results from intracellular recording showed that L-NOARG was without effect on LTP of the e.p.s.p. and, using the extracellular recording technique, it was clear that ODQ in the presence or absence of L-NOARG failed to reduce LTP of the CAP, suggesting that neither NO nor cyclic GMP contributes to LTP in this tissue. These negative results contrast with those reported for the chick ciliary ganglion in which inhibition of NO synthase reduced tetanically-induced LTP (Scott & Bennett, 1993). This same study also found that, as in the SCG, nitroprusside potentiated synaptic transmission. We find, however, that nitroprusside-induced potentiation of synaptic transmission in the SCG persisted under conditions where LTP was saturated, indicating that NO donor-mediated synaptic potentiation is distinct from that occurring in LTP. It remains possible, however, that the pathway may play a transient role in a stage preceding LTP proper, since ODQ (with or without L-NOARG), on average, did cause a small reduction in the CAP amplitude shortly after tetanic stimulation (see Figure 5a, b) although the change was not statistically significant at any time point.

We conclude that, whilst the NO-cyclic GMP pathway is capable of causing a potentiation of neurotransmission in the SCG, we find no evidence that it contributes to LTP. Other mechanisms such as protein kinase C activation (Bachoo *et al.*, 1992) or cyclic AMP accumulation (Briggs *et al.*, 1988; Scott & Bennett, 1993) may instead be more important.

References

ANDO, M., NANBA, T., OKUYA, M. & NAGATA, Y. (1983). Effect of axotomy on the cyclic GMP increase induced by preganglionic stimulation and high extracellular K^+ concentration in superior cervical ganglion of the rat. *Brain Res.*, **267**, 313–321.

BACHOO, B., HEPPNER, T., FIEKERS, J. & POLOSA, C. (1992). A role for protein kinase C in long term potentiation of nicotinic transmission in the superior cervical ganglion of the rat. *Brain Res.*, **585**, 299–302.

BÖHME, G.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.

BOULTON, C.L., SOUTHAM, E. & GARTHWAITE, J. (1995). Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase. *Neuroscience*, **69**, 699–703.

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

BRIGGS, C.A., BROWN, T.H. & MCAFEE, D.A. (1985a). Neurophysiology and pharmacology of long-term potentiation in the rat sympathetic ganglion. *J. Physiol.*, **359**, 503–521.

BRIGGS, C.A. & MCAFEE, D.A. (1988). Long-term potentiation at nicotinic synapses in the rat superior cervical ganglion. *J. Physiol.*, **404,** 129–144.

BRIGGS, C.A., MCAFEE, D.A. & MCCAMAN, R.E. (1985b). Long-term potentiation of synaptic acetylcholine release in the superior cervical ganglion of the rat. *J. Physiol.*, **363,** 181–190.

BRIGGS, C.A., MCAFEE, D.A. & MCCAMAN, R.E. (1988). Long-term regulation of synaptic acetylcholine release and nicotinic transmission: the role of cyclic AMP. *Br. J. Pharmacol.*, **93,** 399–411.

BROWN, T.H. & MCAFEE, D.A. (1982). Long-term synaptic potentiation in the superior cervical ganglion. *Science*, **215,** 1411–1413.

BUSIS, N.A., WEIGHT, F.F. & SMITH, P.A. (1978). Synaptic potentials in sympathetic ganglia: are they mediated by cyclic nucleotides? *Science*, **200,** 1079–1081.

DE VENTE, J., GARSSEN, J., TILDERS, F.J.H., STEINBUSCH, H.W.M. & SCHIPPER, J. (1987). Single cell quantitative immunocytochemistry of cyclic GMP in the superior cervical ganglion of the rat. *Brain Res.*, **411,** 120–128.

DUN, N.J., DUN, S.L., WU, S.Y. & FÖRSTERMANN, U. (1993). Nitric oxide synthase immunoreactivity in rat superior cervical ganglia and adrenal glands. *Neurosci. Lett.*, **158,** 51–54.

GARTHWAITE, J. & BOULTON, C.L. (1995). Nitric oxide signaling in the central nervous system. *Annu. Rev. Physiol.*, **57,** 683–706.

GARTHWAITE, J. & GARTHWAITE, G. (1987). Cellular origins of cyclic GMP responses to excitatory amino acid receptor agonists in rat cerebellum. *J. Neurochem.*, **48,** 29–39.

GARTHWAITE, J., SOUTHAM, E., BOULTON, C.L., NIELSEN, E.B., SCHMIDT, K. & MAYER, B. (1995). Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. *Mol. Pharmacol.*, **48,** 184–188.

LIN, Y.-Q. & BENNETT, M.R. (1994). Nitric oxide modulation of quantal secretion in chick ciliary ganglia. *J. Physiol.*, **481,** 385–394.

MARSH, S. (1989). Extracellular recording from rat sympathetic ganglia and whole nerve bundles. In *Methods in Experimental Physiology and Pharmacology: Biological Measurement Techniques*, Vol. VI. March-Hugstetten: Biomesstechnik.

MORRIS, R., SOUTHAM, E., GITTINS, S.R. & GARTHWAITE, J. (1993). NADPH-diaphorase staining in autonomic and somatic cranial ganglia of the rat. *Neuroreport*, **4,** 62–64.

O'DELL, T.J., HAWKINS, R.D., KANDELL, E.R. & ARANCIO, O. (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.

OKAMURA, H., UMEHARA, K., TADAKI, N., HISA, Y., ESUMI, H. & IBATA, Y. (1995). Sympathetic preganglionic neurons contain nitric oxide synthase and project to the superior cervical ganglion: combined application of retrograde neuronal tracer and NADPH-diaphorase histochemistry. *Brain Res. Bull.*, **5,** 491–494.

SCHUMAN, E.M. & MADISON, D.V. (1991). A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science*, **254,** 1503–1506.

SCOTT, T.R.D. & BENNETT, M.R. (1993). The effect of nitric oxide on the efficacy of synaptic transmission through the chick ciliary ganglion. *Br. J. Pharmacol.*, **110,** 627–632.

SHENG, H., HUGHES, M.L., MURAD, F. & BRIGGS, C.A. (1992). Evidence that nitric oxide mediates the cyclic GMP response to synaptic activity in the rat superior cervical ganglion. *Brain Res.*, **597,** 343–345.

ZHUO, M., HU, Y., SCHULTZ, C., KANDEL, E.R. & HAWKINS, R.D. (1994). Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term potentiation. *Nature*, **368,** 635–639.

(Received April 15, 1996
Revised June 25, 1996
Accepted July 3, 1996)