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Differential effects of nitric oxide donors on basal and electrically evoked release of acetylcholine from guinea-pig myenteric neurones

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1 The effects of the nitric oxide (NO) donors, 3-morpholino-sydnonimine (SIN-1), S-nitroso-N-acetylpenicillamine (SNAP) and sodium nitroprusside on basal and electrically evoked release of [^{3}H]-acetylcholine were studied in myenteric plexus longitudinal muscle preparations of the guinea-pig small intestine preincubated with [^{3}H]-choline.

2 The NO donors concentration-dependently increased basal release of [^{3}H]-acetylcholine. The increase in release was calcium-dependent and was prevented in the presence of tetrodotoxin. Superoxide dismutase (150 u ml^{-1}) potentiated the effect of SIN-1. The selective inhibitor of soluble guanylyl cyclase, 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ, 0.01–1 μM), antagonized the facilitatory effect of SNAP. 8-Bromo cyclic GMP and the cyclic GMP-specific phosphodiesterase inhibitor, zaprinast (both 0.1–1 mM), also enhanced basal [^{3}H]-acetylcholine release. The effect of 10 μM SNAP was significantly enhanced in the presence of zaprinast.

3 The NO donors concentration-dependently inhibited the electrically evoked release of [^{3}H]-acetylcholine, whereas 8-bromo cyclic GMP and zaprinast enhanced the evoked release. The inhibition of acetylcholine release by SNAP was not affected by ODQ (0.01–1 μM).

4 It is concluded that NO stimulates basal acetylcholine release from myenteric neurones through activation of guanylyl cyclase. In addition, NO inhibits the depolarization evoked release of acetylcholine by a presynaptic mechanism unrelated to cyclic GMP. The data imply that NO is not only an inhibitory transmitter to intestinal smooth muscles but also a modulator of cholinergic neurotransmission in the myenteric plexus.

Keywords: Ileum; acetylcholine release; nitric oxide; 3-morpholino-sydnonimine; S-nitroso-N-acetylpenicillamine; sodium nitroprusside; cyclic GMP; zaprinast; ODQ

Introduction

Nitric oxide (NO) is considered as a major non-adrenergic non-cholinergic neurotransmitter causing inhibition of gastrointestinal motility (Sanders & Ward, 1992). Neuronal NO is synthesized by the isoform I of the enzyme NO synthase (see Förstermann *et al.*, 1994) which has been found throughout the gastrointestinal tract in neuronal cell bodies and processes of the myenteric plexus (Bredt *et al.*, 1990). More detailed studies have revealed that about 20% of all myenteric neurones in the guinea-pig small intestine contain constitutive NO synthase immunoreactivity (Costa *et al.*, 1992). These nitrergic neurones innervate not only smooth muscle cells but also make synapses with other myenteric neurones (Llewellyn-Smith *et al.*, 1992) which indicates that NO is either a primary neurotransmitter at neuro-neuronal synapses or modulates the release of other neurotransmitters. Indirect evidence for a modulation by NO of cholinergic neurotransmission has been obtained in biochemical and contraction experiments. NO synthase inhibitors increase the release of acetylcholine evoked by field stimulation of the guinea-pig myenteric plexus (Kilbinger & Wolf, 1994). Moreover, the electrically induced cholinergic contractions of isolated intestinal preparations are enhanced by NO synthase inhibitors (for review see Lefebvre, 1995). All this suggests that endogenous NO, which is released during field stimulation (Wiklund *et al.*, 1993c) inhibits the electrically evoked release of acetylcholine. On the other hand, NO causes an atropine- and tetrodotoxin-sensitive contraction of the non-stimulated guinea-pig ileum, which indicates that NO may increase basal acetylcholine release (Bartho & Lefebvre, 1994).

The aim of the present investigation was to study more directly in overflow experiments the effects of exogenous NO on basal and electrically evoked release of acetylcholine. We have chosen as NO donors, 3-morpholino-sydnonimine (SIN-1), sodium nitroprusside, and S-nitroso-N-acetylpenicillamine (SNAP) which produce NO in biological systems (Feelisch, 1991). Since many effects of NO are mediated via the guanosine 3′:5′-cyclic monophosphate (cyclic GMP) system, the effects of the NO donors were compared with those of the cyclic GMP analogue, 8-bromo cyclic GMP, and with zaprinast which is a cyclic GMP-specific phosphodiesterase inhibitor (for literature see Beavo & Reifsnyder, 1990). In addition, interaction experiments were performed between NO donors and the recently identified selective inhibitor of soluble guanylyl cyclase, 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) (Garthwaite *et al.*, 1995).

A preliminary account of part of this work has been presented to the German Pharmacological Society (Hebeiß & Kilbinger, 1996).

Methods

Guinea-pigs of either sex, weighing 200–400 g, were stunned by a blow to the head and bled. Longitudinal muscle myenteric plexus preparations of the proximal guinea-pig ileum were suspended in a 2 ml organ bath in a physiological salt solution (composition in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, D-glucose 5.6, choline chloride 0.001) at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The preparations were incubated for 30 min with [^{3}H]-choline (2.5 μCi ml^{-1}) during which the tissue was stimulated electrically (0.1 Hz; 1 ms) via two platinum electrodes that

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were positioned parallel to the strips (distance 0.6 cm, voltage drop 10 V cm^{-1}). The strips were then superfused (2 ml min^{-1}) with the physiological salt solution which in addition contained hemicholinium-3, 10 μM. After 60 min washout the superfusate was collected in 3 min fractions and the tritium content of the samples measured by liquid scintillation spectrometry. The strips were stimulated twice at 45 min intervals (S_1/S_2). Biphasic pulses were delivered at a frequency of 10 Hz (1 ms) for 1 min periods from a Grass S6 stimulator.

NO donors, 8-bromo cyclic GMP and zaprinast were added to the superfusate 36 min before S_2. The outflow of tritium evoked by these drugs or by electrical stimulation was calculated from the difference between the total outflow during and after stimulation and the basal outflow of tritium, calculated by interpolation from 2 samples before and after stimulation. At the end of the experiment the tissue was immersed overnight in 3 ml 0.4 M $HClO_4$ and the radioactivity contained in the extract determined. The outflow of tritiated compounds was expressed as a percentage of the radioactivity in the tissue at the beginning of the respective collection period. The effect of drugs on the electrically evoked outflow was calculated by expressing the ratio S_2/S_1 as a percentage of the equivalent ratio obtained in control experiments.

When tritiated acetylcholine and choline were to be determined in the superfusate the strips were superfused after the incubation with [^{3}H]-choline with a medium that contained in addition neostigmine (10 μM). Acetylcholine and choline were extracted from the superfusate by ion-pair extraction with tetraphenylborate/allylcyanide and separated by thin-layer chromatography as described previously (Kilbinger & Nafziger, 1985).

Data analysis

Results are expressed as means ± s.e.mean. The significance of difference between two mean values was assessed by Student's *t* test. For comparison of one control with several experimental groups, the significance of difference was estimated by one-way analysis of variance followed by Dunnett's test.

Drugs

The following were used: [methyl-^{3}H]-choline chloride (80 Ci $mmol^{-1}$; NEN); methylene blue (Merck, Darmstadt, Germany); ODQ (1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one, Tocris Cookson, Bristol, U.K.); SIN-1 (3-morpholinosydnonimine, Cassella, Frankfurt, Germany); SNAP (S-nitroso-N-acetylpenicillamine, RBI, Natick, MA, U.S.A.); superoxide dismutase (Boehringer Mannheim, Germany); 8-bromo cyclic GMP; N^G-nitro-L-arginine; sodium nitroprusside; zaprinast (all from Sigma, St. Louis, MO, U.S.A.); Drugs were dissolved in distilled water or in dimethyl sulphoxide (DMSO) (SNAP, zaprinast). DMSO at 1% (maximum concentration) did not affect basal or electrically evoked outflow of ^{3}H.

Results

Effects of NO donors

The NO donors caused transient increases in basal outflow of ^{3}H. Figure 1a shows as an example the effects of SIN-1. Tetrodotoxin (300 nM) and omission of calcium from the superfusion medium prevented the effects of 300 μM SIN-1 (Figure 2) and of 100 μM SNAP (not shown). In order to identify the nature of the ^{3}H radioactivity in the medium the effect of SIN-1 on basal outflow of ^{3}H was studied in the presence of 10 μM neostigmine, and the labelled acetylcholine

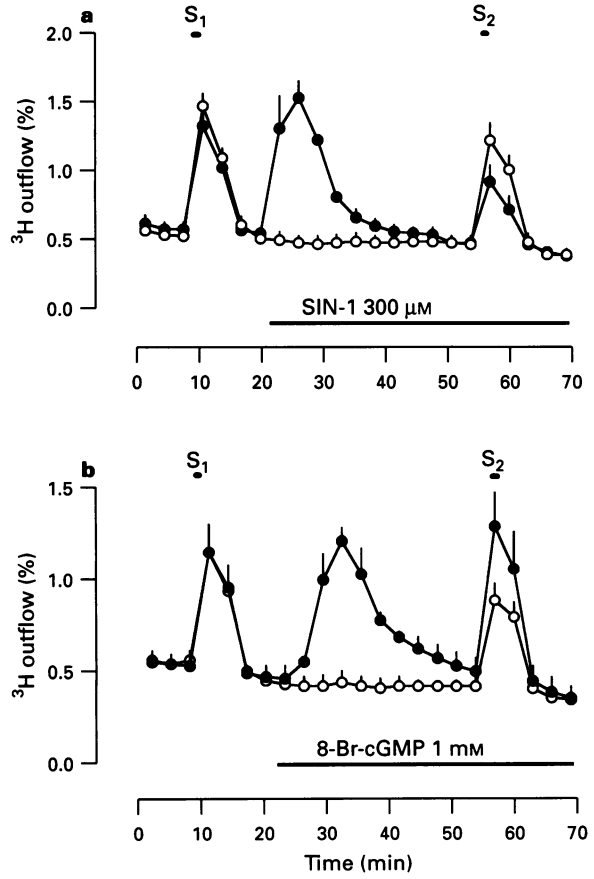

Figure 1 The effects of SIN-1 in the presence of superoxide dismutase (SOD; 150 u ml^{-1}) (a, ●) and of 8-bromo cyclic GMP in the absence of SOD (b, ●) on the outflow of ^{3}H activity from guinea-pig myenteric plexus preparations preincubated with [^{3}H]-choline. SOD was present from 12 min before S_1 onwards. Control experiments (○) without SIN-1 or 8-bromo cyclic GMP were performed either in the presence (a, $n=6$) or absence (b, $n=6$) of SOD. Time 0 min (abscissa scale) corresponds to the end of the 60 min washout period. Ordinate scale, outflow of ^{3}H as percentage of the tritium present in the tissue at the start of each fraction. The preparations were stimulated electrically (S_1, S_2; 10 Hz, 1 min) at 9 and 54 min. Horizontal bars indicate superfusion with SIN-1 ($n=6$) and 8-bromo cyclic GMP ($n=3$). Values are mean with s.e.mean.

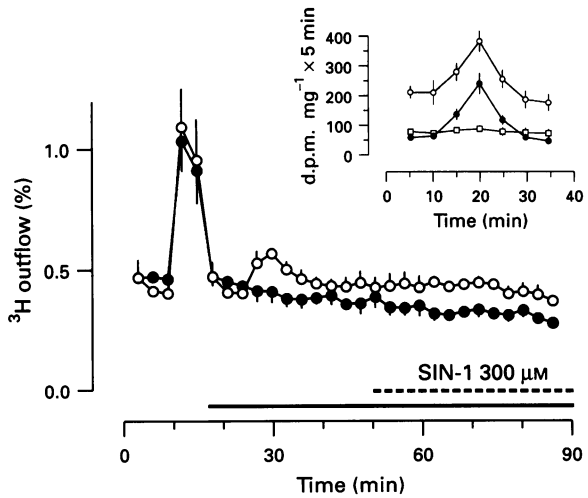

Figure 2 Effects of calcium withdrawal (○; $n=3$) and tetrodotoxin (300 nM; ●; $n=3$) on outflow of ^{3}H elicited by 300 μM SIN-1. The strips were stimulated electrically (10 Hz, 1 min) at 9 min. Calcium was omitted from the medium and tetrodotoxin was added as indicated by the solid horizontal bar. SIN-1 was added as shown by the dotted bar. Mean ± s.e.mean. Inset: Effect of 300 μM SIN-1 on outflow of total ^{3}H radioactivity (○), [^{3}H]-acetylcholine (●) and [^{3}H]-choline (□). The preparations were superfused with SIN-1 from 10 min onwards. Values are mean with s.e.mean ($n=4$).

was separated from labelled choline by thin layer chromatography. Figure 2 (inset) shows that SIN-1 caused an increase in the outflow of [^{3}H]-acetylcholine but not in that of [^{3}H]-choline. The outflow of total ^{3}H elicited by SIN-1 consisted to 94±10% (n=4) of [^{3}H]-acetylcholine. Therefore, the outflow of ^{3}H evoked by the NO donors was taken as a reliable parameter for the release of acetylcholine.

Concentration-response curves for the release-enhancing effect of the NO donors are shown in Figure 3a and c. The maximal increases in outflow were comparable for the three compounds (2.3–2.7% of the tritium tissue content). In contrast to SNAP and sodium nitroprusside, SIN-1 generates not only NO but also superoxide anions which reduce the half-life of released NO (Feelisch *et al.*, 1989). The effects of SIN-1 were therefore studied in the presence of superoxide dismutase (SOD). SOD (150 u ml^{-1}) added 12 min before S_1 did not affect the ^{3}H outflow evoked by S_1, but potentiated the effects of SIN-1 on basal outflow (Figure 3). On the other hand, the effect of 100 μM SNAP on basal outflow was not significantly changed in the presence of SOD (see Figure 5c). Sodium nitroprusside, SIN-1 (plus SOD) and SNAP were approximately equipotent in increasing basal ^{3}H outflow.

The electrically evoked ^{3}H outflow was diminished by the NO donors (Figure 3b, d). However, significant inhibitions were not observed until concentrations of 100 μM or higher. The inhibition of release by SIN-1 was stronger in the presence than in the absence of SOD. SNAP was about 10 times more potent than the two other compounds.

Effects of 8-bromo cyclic GMP and zaprinast

8-Bromo cyclic GMP (see Figure 1) and zaprinast concentration-dependently enhanced basal outflow of ^{3}H (Figure 4a), zaprinast being about 10 times more potent than 8-bromo cyclic GMP. The increases in basal outflow caused by 300 μM 8-bromo cyclic GMP and 100 μM zaprinast were abolished by omission of calcium from the medium (n=2 for each drug; not shown). Consideration of expense became a limiting factor at high concentrations of 8-bromo cyclic GMP, and the maximal effect on basal outflow was therefore not determined. The increase by 100 μM zaprinast of basal outflow (2.15±0.33%, n=6) was significantly reduced (to 1.33± 0.12%, n=6; P<0.05) in the presence of 100 μM N^G-nitro-L-arginine.

Both 8-bromo cyclic GMP and zaprinast also enhanced the electrically evoked ^{3}H outflow, and 8-bromo cyclic GMP was about 10 times more potent than zaprinast (Figure 4b).

The influence of zaprinast on the increase by SNAP of basal outflow was studied in separate experiments. Strips were stimulated electrically (only S_1, 10 Hz, 1 min) and 12 min later SNAP was added to the superfusate. In the presence of zaprinast (100 μM; added 36 min before SNAP) the ^{3}H outflow evoked by 10 μM SNAP (0.50±0.14%, n=5) was significantly increased to 1.01±0.15% (n=8; P<0.05).

Influence of ODQ and methylene blue on the effects of SNAP on acetylcholine release

The effects of 100 μM SNAP were tested in the presence of the guanylyl cyclase inhibitor ODQ. In control experiments without SNAP, ODQ changed neither the electrically evoked ^{3}H outflow during S_1 nor the ratio S_2/S_1 (Table 1). Figure 5a shows that ODQ strongly inhibited the increase of basal ^{3}H outflow by SNAP. A concentration of only 10 nM reduced significantly the facilitatory effect of the NO donor. On the

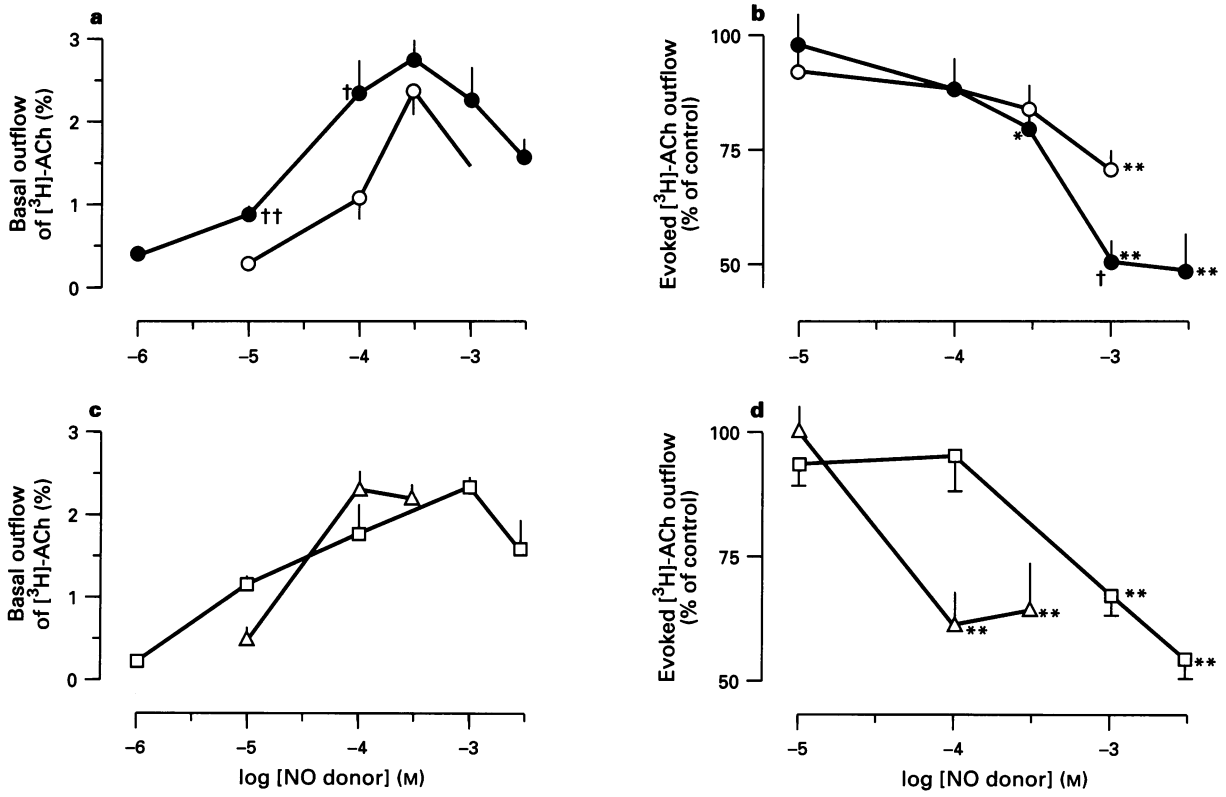

Figure 3 Increase of basal outflow (a, c) and inhibition of electrically evoked outflow (b, d) of [^{3}H]-acetylcholine by SIN-1 in the absence (○) and presence (●) of superoxide dismutase (SOD 150 u ml^{-1}), by sodium nitroprusside (□), and SNAP (△). Strips were stimulated twice (10 Hz, 1 min) (S_1, S_2) and the NO donors were added 36 min before S_2. Control experiments were run in parallel, and the effects of the NO donors on the evoked outflow are given as a percentage of the corresponding control value. Basal outflow is given as a percentage of the amount of tritium in the tissue at the start of superfusion with the NO donor. Values are mean with s.e.mean of 4–8 experiments. Significance of inhibition of [^{3}H]-acetylcholine outflow: *P<0.05, **P<0.01. Significance of the effect of SOD: †P<0.05, ††P<0.01.

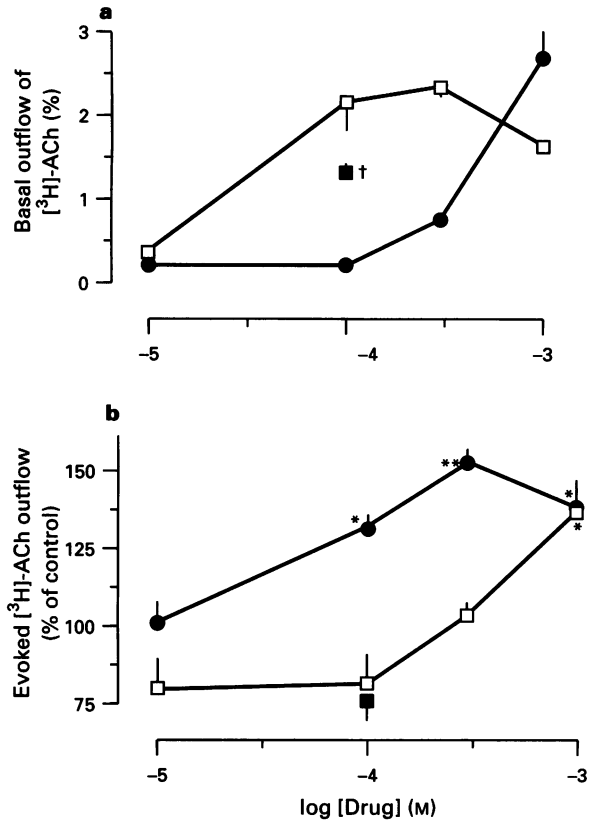

Figure 4 Concentration-response curves for the effects of 8-bromo cyclic GMP (●) and of zaprinast [in the absence (□) and presence (■) of N^G-nitro-L-arginine] on basal and electrically evoked outflow of [^{3}H]-acetylcholine. Strips were stimulated twice (S_1, S_2) and zaprinast or 8-bromo cyclic GMP were added 36 min before S_2. N^G-nitro-L-arginine (100 μM) was superfused from 30 min before S_1 onwards. Values are mean with s.e.mean of 4–6 experiments except experiments with 1 mM 8-bromo cyclic GMP where $n=3$. Significance of increase in the evoked [^{3}H]-acetylcholine outflow: $^*P<0.05$, $^{**}P<0.01$. Significance of difference to the effect of zaprinast alone: $\dagger P<0.05$.

Table 1 Effects of ODQ on electrically evoked outflow of [^{3}H]-acetylcholine from myenteric plexus preparations

ODQ (nM)	S_1 (%)	S_2/S_1	n
–	1.35±0.21	0.85±0.06	8
10	0.83±0.10	0.87±0.07	4
100	0.76±0.10	1.06±0.04	4
1000	1.48±0.26	0.92±0.04	4

After preincubation with [^{3}H]-choline the preparations were superfused and electrically stimulated twice (S_1,S_2). ODQ was present from 24 min before S_1 until the end of the experiment. The outflow evoked by S_1 is given as percentage of the tritium content in the tissue at the start of S_1. There was no statistically significant difference between the S_1 values and between the S_2/S_1 ratios. Mean±s.e.mean.

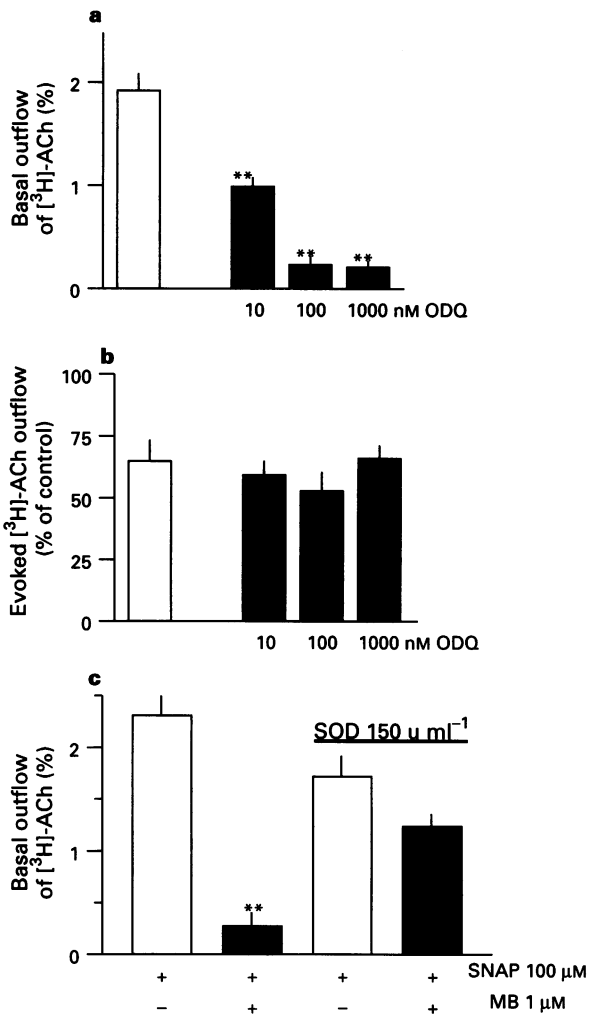

Figure 5 ODQ antagonizes the NO-mediated increase of basal outflow (a), but not the inhibition of the evoked outflow of [^{3}H]-acetylcholine (b). Strips were stimulated twice (10 Hz, 1 min) (S_1, S_2). ODQ was added to the superfusate 24 min before S_1 and SNAP (100 μM) 36 min before S_2. The effects of SNAP on the evoked outflow are given as a percentage of the corresponding control values given in Table 1. Open columns, effects of SNAP on basal and electrically evoked ^{3}H outflow ($n=6$). Solid columns, effects of SNAP in the presence of 10–1000 nM ODQ ($n=4$ each). Mean±s.e.mean. $^{**}P<0.01$. (c) Effects of methylene blue (MB, 1 μM) and methylene blue plus superoxide dismutase (SOD, 150 u ml^{-1}) on the increase by SNAP (100 μM) of basal [^{3}H]-acetylcholine outflow. Methylene blue or methylene blue plus superoxide dismutase were added to the superfusate 32 min before SNAP. Values are mean with s.e.mean; $n=4$–8. Significance of difference from outflow in the absence of methylene blue: $^{**}P<0.01$.

other hand, ODQ did not affect the inhibition by SNAP of the evoked outflow (Figure 5b).

Methylene blue, like ODQ, strongly attenuated the release-enhancing action of 100 μM SNAP (Figure 5c). However, since methylene blue may generate superoxide anion and thus inactivate NO (Mayer *et al.*, 1993), the effect of SNAP on basal outflow was tested in the presence of methylene blue plus superoxide dismutase (150 u ml^{-1}). Figure 5c shows that in the presence of superoxide dismutase the release-enhancing effects of SNAP were not significantly different in the absence and presence of methylene blue.

Discussion

Basal release

The increase by NO donors of basal [^{3}H]-acetylcholine release was prevented by tetrodotoxin and was calcium-dependent. This indicates that the enhanced release is not due to a cytotoxic action on cholinergic neurones, but that the NO donors evoke an exocytotic release of acetylcholine. A similar enhancement of basal acetylcholine release by various NO donors was found in different parts of the brain both *in vivo*

(Prast & Philippu, 1992; Guevara-Guzman *et al.*, 1994; Prast *et al.*, 1995) and *in vitro* (Lonart *et al.*, 1992; Ohkuma *et al.*, 1995).

Which mechanisms are involved in the effect of NO donors? For the following reasons we suggest that the increase in acetylcholine release can be attributed to the activation by NO of guanylyl cyclase in myenteric neurones: Firstly, ODQ, in concentrations which selectively inhibit soluble guanylyl cyclase (Garthwaite *et al.*, 1995), antagonized the NO donor-mediated enhancement of acetylcholine release. Methylene blue was used as an additional tool to demonstrate the involvement of guanylyl cyclase, since low concentrations of methylene blue may inhibit soluble guanylyl cyclase (see Mayer *et al.*, 1993). In the present study methylene blue reduced the increase by SNAP of acetylcholine release. However, superoxide dismutase prevented the antagonistic effect of methylene blue which may generate superoxide anion and thus inactivate NO (Mayer *et al.*, 1993). Hence, methylene blue probably inactivates NO via generation of superoxide anion, but does not function as an inhibitor of the soluble guanylyl cyclase (see also Marczin *et al.*, 1992). Secondly, 8-bromo cyclic-GMP, the cell-permeable analogue of cyclic GMP, increased concentration-dependently basal release. A comparable finding has previously been described by Matusak *et al.* (1991). Zaprinast, which specifically inhibits the breakdown of cyclic GMP (Beavo & Reifsnyder, 1990) similarly enhanced basal release of acetylcholine. The effect of zaprinast was diminished by N^G-nitro-L-arginine which indicates that there is a continuous synthesis and release of endogenous NO which activates guanylyl cyclase. Thirdly, inhibition of cyclic GMP hydrolysis by zaprinast significantly enhanced the effect of 10 μM SNAP on basal release. This also illustrates that a cyclic GMP pathway is involved in the release-enhancing action of the NO donors.

A recent study suggests that peroxynitrite mediates the stimulatory effect of NO (Ohkuma *et al.*, 1995). NO can react with superoxide to yield peroxynitrite which may alter membrane functions. In support of this hypothesis, exogenous peroxynitrite enhanced the release of acetylcholine from mouse cerebral cortex and superoxide dismutase diminished the stimulatory effects of NO donors (Ohkuma *et al.*, 1995). However, this explanation can be excluded for the present experiments, because superoxide dismutase potentiated the release-enhancing effect of SIN-1.

The site of action of NO is probably the cholinergic cell bodies within myenteric ganglia, because the increase in release involved action potential propagation, as shown by its blockade by tetrodotoxin. Guanylyl cyclase has been identified in neurones of myenteric ganglia, and exogenous NO or electrical field stimulation in the presence of zaprinast cause an accumulation of cyclic GMP-like immunoreactivity in these neurones (Shuttleworth *et al.*, 1993). The enhancement by NO of acetylcholine release is paralleled by its effect on intestinal smooth muscle. Exogenous NO causes a contraction of the longitudinal muscle of guinea-pig ileum which is prevented by atropine and tetrodotoxin (Bartho & Lefebvre, 1994).

Evoked release

Wiklund *et al.* (1993a) have shown that NO (formed from 100 μM sodium nitrite in hydrochloric acid) caused a slight but significant inhibition of the electrically evoked [^{3}H]-acetylcholine release from guinea-pig myenteric plexus. The present experiments confirm and extend this finding. The mechanism of the NO mediated inhibition of acetylcholine release remains to be clarified. Cyclic GMP is certainly not involved, because ODQ did not affect the inhibition by SNAP of the evoked release. This interpretation is supported by the fact that 8-bromo cyclic GMP and zaprinast did not reduce, but enhanced the evoked release.

The site of action of NO may be the nerve terminal, since a presynaptic inhibitory action of sodium nitroprusside was also found in electrophysiological experiments on guinea-pig myenteric neurones (Tamura *et al.*, 1993). In this study sodium nitroprusside suppressed the non-cholinergic slow excitatory postsynaptic potential (e.p.s.p.), but not the fast e.p.s.p. at nicotinic synapses. It is thus possible that the NO-mediated inhibition of acetylcholine release is secondary to the inhibition of the release of another neurotransmitter which stimulates cholinergic neurones via slow excitatory synapses.

In the present study only high concentrations of the NO donors (100 μM and more) inhibited the evoked release of acetylcholine. This contrasts with the effects of NO donors on guinea-pig intestinal smooth muscle where concentrations of 1–10 μM already cause significant relaxations (Osthaus & Galligan, 1992). Smooth muscle relaxation is, however, due to the activation by NO of guanylyl cyclase (Sanders & Ward, 1992), and it is conceivable that the cyclic GMP pathway is more sensitive to the effects of NO than the unknown pathway in neurones which leads to inhibition of acetylcholine release. That the inhibition by NO of acetylcholine release may have physiological significance is evident from previous studies which have shown that NO synthase inhibitors facilitate the depolarization-evoked release of acetylcholine from guinea-pig myenteric plexus (Kilbinger & Wolf, 1994) and canine intestinal circular muscle (Hryhorenko *et al.*, 1994). These data imply that endogenous NO exerts a tonic presynaptic inhibition of acetylcholine release. The release experiments are corroborated by contraction studies: sodium nitroprusside depressed the cholinergic contraction elicited by electrical stimulation of gastric smooth muscle, but did not influence the contraction caused by exogenous acetylcholine (Baccari *et al.*, 1994). *Vice versa*, inhibitors of NO synthase enhanced the electrically induced cholinergic contractions of guinea-pig ileum and taenia coli (Knudsen & Tottrup, 1992; Wiklund *et al.*, 1993b), and of rat and rabbit stomach (Lefebvre *et al.*, 1992; Baccari *et al.*, 1993). All this suggests that NO is able to inhibit the cholinergic motor neurotransmission in the gastrointestinal tract. Although the predominant effect of NO in the intestine is certainly the direct relaxation of smooth muscle, the reduction of acetylcholine release may be an additional inhibitory mechanism that is mediated by a different pathway.

Conclusion

Our results suggest that NO inhibits, presynaptically, the depolarization-evoked release of acetylcholine via a mechanism which is unrelated to cyclic GMP. Moreover, NO stimulates cholinergic cell bodies via activation of guanylyl cyclase which leads to an increase in basal acetylcholine release. Comparable dual effects of drugs on acetylcholine release from myenteric neurones have repeatedly been reported in the literature. Agonists such as 5-hydroxytryptamine, substance P, GABA, and muscarine enhance basal release via stimulation of somatodentritic receptors, namely 5-HT_3-, NK_3-, $GABA_A$- and M_1-receptors, respectively. In addition, these drugs inhibit the depolarization evoked release of acetylcholine via stimulation of different subtypes of presynaptic receptors, i.e. 5-HT_{1A}-, NK_1-, $GABA_B$-, M_3-receptors (for review, see Fuder & Muscholl, 1995). The biphasic modulation by NO donors of acetylcholine release as presented in this paper is in agreement with recent observations of a NO donor-mediated increase of basal and inhibition of evoked release of dopamine from PC12 cells (Sun *et al.*, 1995), and the facilitation and inhibition by SIN-1 of acetylcholine release at cholinergic synapses of *Aplysia* (Meulemans *et al.*, 1995). Taken together, our data imply that NO is not only a primary transmitter to intestinal smooth muscle but also a modulator of cholinergic neurotransmitter in the myenteric plexus.

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