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Effects of nitric oxide donors on basal and K^+-evoked release of [^{3}H]noradrenaline from rat cerebral cortex synaptosomes

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Abstract

We investigated the effects of nitric oxide (NO) donors, *S*-nitroso-*N*-acetylpenicillamine and sodium nitroprusside on basal and K^+-evoked release of [^{3}H]noradrenaline from superfused synaptosomes from the rat cerebral cortex. Both substances produced concentration-dependent increases in the release of the labeled transmitter under basal and depolarized conditions. The effects of the donors on basal release were Ca^{2+}-independent but were not inhibited by the carrier-uptake blocker, desipramine; the effects were abolished by hemoglobin (an NO scavenger). Thirty-five minutes after stimulation with sodium nitroprusside, the synaptosomes were still responsive to KCl stimulation, indicating that the donor's effects were not caused by damage to the synaptosome membrane. The cGMP analogue, 8-bromo-cGMP, had no effect on basal release, and the enhanced release produced by sodium nitroprusside was not inhibited by the specific inhibitor of soluble guanylate cyclase, 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one, indicating that NO's effects on basal release of the neurotransmitter are guanylate cyclase-independent. Both of the NO donors had more marked effects on release of [^{3}H]noradrenaline during K^+-stimulated depolarization. The NO-mediated increase in this case was partially antagonized by 10 μM 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one, and 8-Br-cGMP was also capable of producing concentration-dependent increases in the K^+-stimulated release of the transmitter. These findings indicate that the effects of the NO donors on [^{3}H]noradrenaline release during depolarization are partially mediated by the activation of guanylate cyclase.

Keywords: Synaptosomes, superfused; [^{3}H]Noradrenaline release; *S*-Nitroso-*N*-acetylpenicillamine; Sodium nitroprusside; cGMP; 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one

1. Introduction

Initially identified as a mediator used by macrophages and endothelial cells, nitric oxide (NO) is now recognized as a prominent neuronal messenger (Garthwaite, 1991; Bredt and Snyder, 1992). NO is thought to play a number of physiological roles within the central nervous system including long-term potentiation (Bliss and Collingridge, 1993), long-term depression (Zorumski and Izumi, 1993), and neuroprotection (Lipton et al., 1993). Several investigators have suggested that NO acts as a retrograde messenger produced by the postsynaptic neuron to control the release of neurotransmitters by presynaptic terminals (O'Dell et al., 1991; Schuman and Madison, 1991; Fazeli, 1992). More recent studies have in fact demonstrated NO-induced increases in the release of adenosine, acetylcholine, noradrenaline and glutamate from hippocampal slices (Fallahi et al., 1996; Lauth et al., 1995; Lonart and Johnson, 1995a,b; Satoh et al., 1996), as well as that of glutamate from hippocampal synaptosomes (Meffert et al., 1994). Excitatory and inhibitory effects on neurotransmitter release in other areas of the brain have also been observed (Stewart et al., 1996; Prast and Philippu, 1992; Black et al., 1994; Bugnon et al., 1994; Guevara-Guzman et al., 1994; Lonart and Johnson, 1994; Seilicovich et al., 1995; Zhu and Luo, 1992).

The present study was conducted to assess the effects of NO on the release of noradrenaline by synaptosomes isolated from the cerebral cortex of the rat. Cortical tissue is also known to contain high levels of NO synthase, the enzyme that is responsible for the formation of NO. Investigation of cortical NO activity has been relatively limited, although the gas appears to play an important role in the development and function of this tissue (Vincent and Hope, 1992). The synaptosome model allowed us to focus

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our attention on the mechanisms that regulate presynaptic neurotransmitter release minimizing the interference by other brain structures.

The superfused synaptosomes were exposed to sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine, which produce NO in biological systems (Feelisch, 1991), and the release of [^{3}H]noradrenaline was measured under resting conditions and during K^+-stimulated depolarization. Since many of the effects of NO are mediated by the guanosine 3′,5′-cyclic monophosphate (cGMP) system, the effects of the NO donors were also compared with those produced by the cell-permeable cGMP analogue, 8-bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP), and attempts were made to block their effects using a selective inhibitor of soluble guanylate cyclase, 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (Garthwaite et al., 1995).

2. Materials and methods

2.1. Preparation and superfusion of synaptosomes

Adult male Wistar rats (180–200 g) were sacrificed by decapitation and the brains rapidly removed. Crude cortical synaptosome fractions (P_2) were prepared according to the method of Gray and Whittaker (1962). Using a glass-Teflon grinder, the tissue was homogenized in 40 volumes of 0.32 M sucrose that had been buffered to pH 7.4 with phosphate. The homogenate was centrifuged (5 min, $1000 \times g$), and the resulting supernatant was recentrifuged (20 min, $12{,}000 \times g$) to isolate the synaptosomes. The pellet obtained was resuspended in a physiological medium (millimolar composition: NaCl, 125; KCl, 3; $MgSO_4$, 1.2; $CaCl_2$, 1.2; NaH_2PO_4, 1.0; $NaHCO_3$, 22, glucose, 10—pH 7.2–7.4) aerated with 95% O_2/5% CO_2 at 37°C. The protein content was determined as described by Lowry et al. (1951) using bovine serum albumin as a standard. The synaptosome suspension was then incubated with [^{3}H]noradrenaline (final concentration 0.05 μM) for 15 min at 37°C in an atmosphere of 95% O_2/5% CO_2. After labeling, aliquots of the suspension were layered (0.8–1 mg of protein in the different experiments) on to Millipore filters at the bottom of 20 parallel superfusion chambers (Raiteri et al., 1974) and superfused with a continuously aerated medium (95% O_2/5% CO_2) at 0.6 ml/min. After an initial 38-min equilibration period, during which spontaneous outflow reached a constant rate, the NO-donors or 8-Br-cGMP were added. Exposure time was 5 min, after which the perfusion was continued with standard medium. All experiments using sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine were performed with solution containers and tubing protected by aluminum foil. In experiments using 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one, the inhibitor was added after the equilibration period and 8 min prior to the addition of the NO-donors. In experiments using desipramine, the superfusion medium in all the chambers contained 10 μM of the noradrenaline uptake-blocker throughout the experiment. In some experiments, a Ca^{2+}-free medium was used throughout the period of superfusion. Experiments were also performed in the presence of hemoglobin prepared as described by Martin et al. (1985). A 1 mM solution of commercially available hemoglobin was prepared in distilled water and combined with a 10-fold molar excess of sodium dithionite. The latter was subsequently removed by 4 h of dialysis in 1000 volumes of distilled water at 4°C. Aliquots of the solution were stored for up to 7 days at −20°C. To evaluate the effects of NO on release of the neurotransmitter during depolarization, sodium nitroprusside, *S*-nitroso-*N*-acetylpenicillamine, or 8-Br-cGMP were added to the medium together with 9 mM KCl (after removal of an equimolar concentration of NaCl). Exposure time was 5 min. The various experimental conditions are described in the figure legends. Fractions of the perfusate were collected at 2 or 5 min intervals (depending on total perfusion time), using vials containing 100 μl of a protective solution (1.5% EDTA, 1% ascorbic acid, 0.001% unlabelled noradrenaline).

An aliquot of the collected perfusate fraction was counted by liquid scintigraphy for evaluation of total radioactivity; another aliquot of the same fraction was analyzed for its content of [^{3}H]noradrenaline according to the method of Smith et al. (1975). After separation of the [^{3}H]-deaminated metabolites on Bio-Rex 70 columns, the [^{3}H]noradrenaline present in each fraction was measured using liquid scintigraphy. Measurement of the total radioactivity and of the amine radioactivity did not appeared different in percentage in the various experimental conditions.

2.2. Data analysis

The amount of [^{3}H]noradrenaline found in each superfusate fraction was calculated as the percentage of total [^{3}H]noradrenaline recovered (fractions plus filter). Release curve were obtained by plotting the percentage of [^{3}H]noradrenaline in each collected fraction against perfusion time (in minutes), and the integrated area under the curve was used to evaluate the response to each test agent. Curve parameter were evaluated by the GraphPad program. Perfusion time values were plotted with a 2-min delay since it takes about 2 min for the fluid to flow from the filters to the collecting vials.

All results were expressed as means ± S.E.M. The significance of the difference between two mean values was determined with the Student *t*-test. Comparison of a single control value with those emerging from several experimental groups was based on one-way analysis of variance followed by Dunnett's test. $P < 0.05$ was considered significant.

2.3. Drugs

The following were used: L-[7,8-^{3}H]noradrenaline (37 Ci/mmol; Amersham, Buckinghamshire, UK); desipramine and sodium nitroprusside (Sigma; St. Louis, MO, USA); *S*-nitroso-*N*-acetylpenicillamine, (1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one), 8-bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP) from Alexis, Läufelfingen, Switzerland. Drugs were dissolved in distilled water or in dimethyl sulphoxide (*S*-nitroso-*N*-acetylpenicillamine). Dimethyl sulphoxide at 1% (maximum concentration) did not affect basal or K^+-evoked outflow of [^{3}H]noradrenaline.

3. Results

3.1. Effects of nitric-oxide donors on basal release of [^{3}H]noradrenaline from rat cortical synaptosomes

The synaptosomes were exposed for 5 min to sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine (both at concentration of 0.01–10 mM) to identify the possible effects of NO on basal release of [^{3}H]noradrenaline. As shown in Fig. 1, both drugs caused dose-dependent increases in the release of the labeled neurotransmitter. The maximum release induced by sodium nitroprusside was

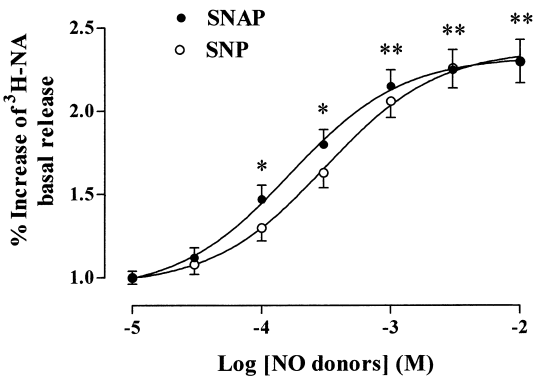

Fig. 1. Effects of sodium nitroprusside (SNP) and *S*-nitroso-*N*-acetylpenicillamine (SNAP) on outflow of [^{3}H]noradrenaline from rat cortical synaptosomes in the resting state. Synaptosomes were labeled with 0.05 μM [^{3}H]noradrenaline and superfused as described in Section 2. After a 38-min equilibration period, the synaptosomes were exposed for 5 min to the NO-donors, sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine. Perfusion was then resumed with standard medium. Fractions were collected every 2 min and analyzed for [^{3}H]noradrenaline. The amount in each fraction was expressed as the percentage of total [^{3}H]noradrenaline recovered (fractions plus filter). Fraction percentages were plotted against perfusion time (minutes) and the integrated area under the curve was used to evaluate the response to each agent. Values have been plotted with a 2-min delay, which reflects the fraction collection process itself (i.e., period during which the medium was flowing through the tubules into the collecting vials). Results in the figure are expressed as percentage increases in basal release of [^{3}H]noradrenaline. Each point represents the mean ± S.E.M. of four experiments run in triplicate. $*\ P < 0.05$; $**\ P < 0.001$ versus basal levels.

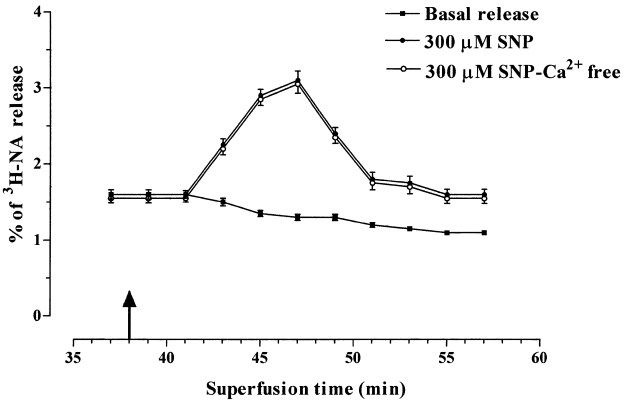

Fig. 2. Effect of Ca^{2+}-deprivation on [^{3}H]noradrenaline release elicited by 300 μM sodium nitroprusside. After 38 min of equilibration, synaptosomes were exposed to 300 μM sodium nitroprusside in perfusion medium with or without Ca^{2+} ions. In the latter case, Ca^{2+}-free medium was used throughout the entire perfusion period. The arrow indicates the moment of the application of the stimulus. Release of [^{3}H]noradrenaline peaked at the 47th min of perfusion, i.e., 9 min after addition of sodium nitroprusside (see legend to Fig. 1). For other experimental details, see Section 2 and the legend to Fig. 1. Each point represents the mean ± S.E.M. of three experiments run in triplicate. Where not shown in the figure, the S.E.M. values were smaller than symbols.

2.37 times higher than that observed at baseline, and the ED_{50} was 300 ± 15 μM; $n = 4$. The ED_{50} for *S*-nitroso-*N*-acetylpenicillamine (180 ± 9 μM; $n = 4$) was somewhat lower than that of sodium nitroprusside, but the maximal effects of the two NO donors on transmitter release were similar. The release response to sodium nitroprusside (Fig. 2) and *S*-nitroso-*N*-acetylpenicillamine (data not shown) peaked after approximately 9 min of exposure; the effects of the NO-donors were totally reversible at all the concentrations tested.

The stimulatory effect of sodium nitroprusside was not dependent on the presence of Ca^{2+} in the medium (Fig. 2); the same was true of *S*-nitroso-*N*-acetylpenicillamine. Exposure to cyanide derivatives of iron (300 μM) $K_3Fe(CN)_6$ or $K_4Fe(CN)_6$ had no effect whatsoever on either basal or K^+-stimulated release of the neurotransmitter (data not shown), thus excluding the possibility that the effects of sodium nitroprusside were due to the release of cyanide ions.

[^{3}H]Noradrenaline release induced by the NO donors was not modified by exposure to the noradrenaline-uptake blocker, desipramine: the addition to the superfusion medium of 10 μM desipramine does not modify the effects of sodium nitroprusside at all the concentrations tested (data not shown).

Sodium nitroprusside-induced enhancement of [^{3}H]noradrenaline release was significantly inhibited by co-perfusion with hemoglobin (20 μM), and the latter had no effect on release when administered alone (Fig. 3). When a KCl (9 mM) stimulus was delivered after exposure to sodium nitroprusside (300 μM), the release observed was

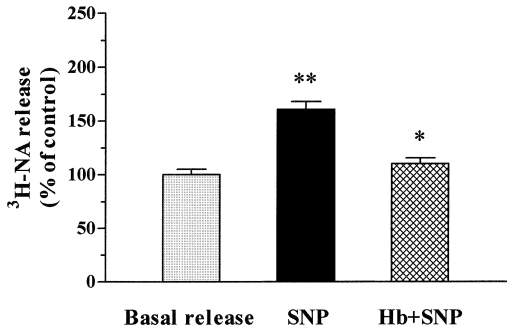

Fig. 3. Effect of the NO scavenger hemoglobin on [^{3}H]noradrenaline release elicited by 300 μM sodium nitroprusside. After 30 min of perfusion with standard medium, 20 μM hemoglobin was added and perfusion continued for 8 min. To the medium containing hemoglobin, 300 μM sodium nitroprusside was then added and left for 5 min. Hemoglobin reduced the increment induced by sodium nitroprusside by 83%. Each column represents the mean ± S.E.M. of three experiments run in triplicate. $**P < 0.01$ versus basal levels; $*P < 0.01$ versus sodium nitroprusside treatment.

no different from that evoked by two subsequent stimulations with 9 mM KCl alone (Fig. 4), which tends to rule out the possibility that the effects of sodium nitroprusside are related to damage to the cell itself and/or its secretory mechanisms.

Since NO is known to stimulate soluble guanylate cyclase and enhance cGMP levels, the cell-permeable cGMP analogue, 8-Br-cGMP, was tested to see if it mimicked the effect of the NO-generating compounds. Unlike sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine, 8-Br-cGMP had no effect on basal [^{3}H]noradrenaline release from synaptosomes. When the synaptosomes were exposed to sodium nitroprusside (300 μM) in the presence of the specific guanylate-cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (10 μM), outflow of the transmitter was the same as that observed with sodium nitroprusside alone (data not shown).

3.2. Effects of nitric-oxide donors on release of [^{3}H]noradrenaline from synaptosomes during K$^+$-stimulated depolarization

Both of the NO donors also caused dose-dependent increases in the release of [^{3}H]noradrenaline induced by 9 mM KCl (Fig. 5). In both cases, the effects were more significant than those exerted on basal release, with a 3.28-fold increase in K$^+$-stimulated release at maximal doses. Again, the ED_{50} for *S*-nitroso-*N*-acetylpenicillamine (115 ± 6 μM, $n = 4$) was lower than that of sodium nitroprusside (200 ± 10 μM, $n = 4$).

As shown in Fig. 6 the increase induced by the NO-donors appeared immediately after the application of the stimulus despite of what observed with the basal release. In this case as well, the enhancement was inhibited by co-perfusion with 20 μM hemoglobin, indicating that this effect can be attributed to the formation of NO (Fig. 7).

There were some differences between the stimulatory effects of the NO donors on K$^+$-evoked vs. basal release of the neurotransmitter. The increase produced by 300 μM sodium nitroprusside (2.03 ± 1.03 times that seen with KCl alone) was significantly reduced by pretreatment with

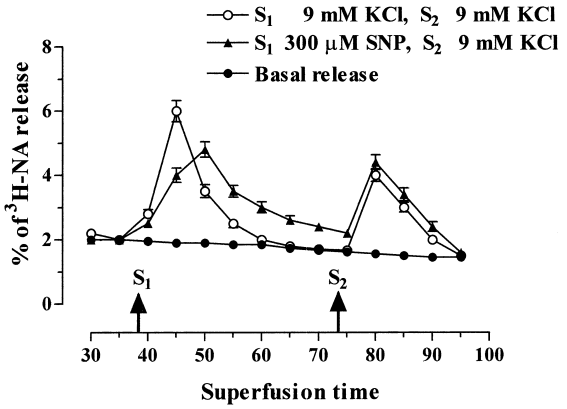

Fig. 4. Reversibility of the effects of sodium nitroprusside on basal release of [^{3}H]noradrenaline. In these experiments, synaptosomes were exposed to two subsequent stimulations (S_1 and S_2); the arrows indicate the timing of the application of the two stimuli. Fractions of the perfusate were collected every 5 min due to the longer perfusion period. After 38 min of equilibration, in one experimental condition (○) the first stimulus was 9 mM KCl while in the other (▲) first stimulus was 300 μM sodium nitroprusside. Thirty-five minutes after the application of the first stimulus, the synaptosomes were exposed for 5 min to a medium containing 9 mM KCl in both the experimental condition. The treatment with sodium nitroprusside does not modify the response to the second KCl stimulation. Curves are based on means ± S.E.M. of three experiments run in triplicate.

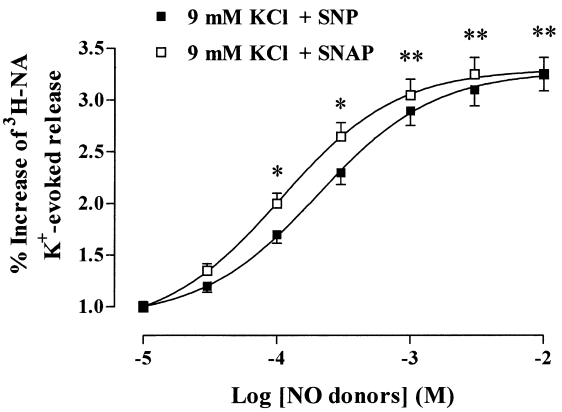

Fig. 5. Effects of sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine (SNAP) on [^{3}H]noradrenaline release during K$^+$-stimulated depolarization. After a 38-min equilibration period, the synaptosomes were exposed for 5 min to a medium containing 9 mM KCl, with or without sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine at various concentrations. The NO donors increased the release of the neurotransmitter stimulated by depolarization. The percentages of total [^{3}H]noradrenaline released in each collected fraction were plotted against perfusion time (minutes) to obtain the curves. Area under the curve was calculated and the values obtained were used to calculate the increase produced by the NO donors. Each point represents the mean ± S.E.M. of four experiments run in triplicate. $*P < 0.05$; $**P < 0.001$ versus K$^+$-stimulated release.

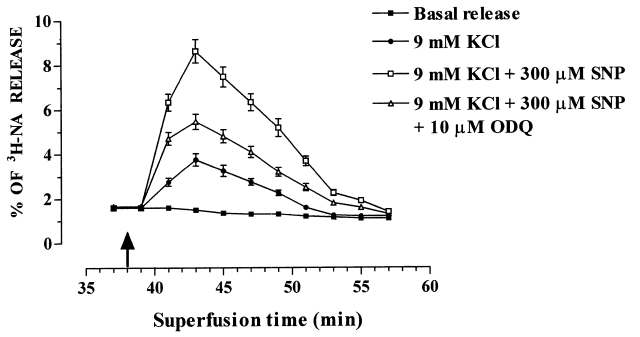

Fig. 6. Partial antagonism by 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) of the NO-mediated increase in K^+-stimulated release of [^{3}H]noradrenaline. After 30 min equilibration, 10 μM 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one was added to the perfusion medium. Eight minutes later, the synaptosomes were stimulated with 9 mM KCl plus 300 μM sodium nitroprusside. The 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one reduced the increment induced by 300 μM sodium nitroprusside by 60%. Each point represents the mean ± S.E.M. of four experiments run in triplicate.

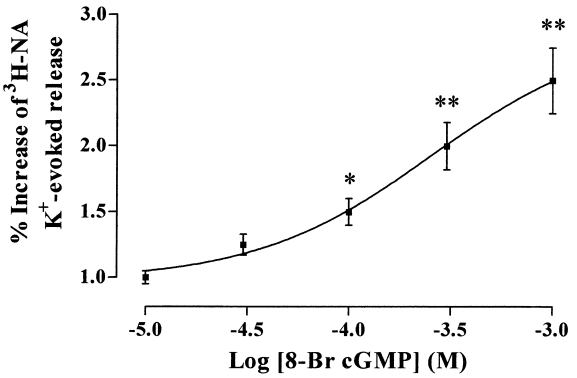

Fig. 8. Concentration–response curve showing the effect of 8-Br-cGMP on K^+-stimulated release of [^{3}H]noradrenaline. After 38 minutes of equilibration, synaptosomes were depolarized with 9 mM KCl (5 min), with or without 8-Br-cGMP (five different concentrations). The percentage increase in K^+-stimulated release of [^{3}H]noradrenaline induced by the analogue was calculated from the area under the concentration–response curve. Values are means ± S.E.M. of four different experiments. $*\ P < 0.05$; $**\ P < 0.001$ versus K^+-stimulated release.

1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (10 μM) (1.43 ± 0.75 times that of KCl alone) (Fig. 6). According to Garthwaite et al. (1995) the maximal inhibition of guanylate cyclase activity by 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one was obtained with 1 μM concentration.

This finding is consistent with our observation of dose-dependent enhancement of K^+-stimulated release produced by 8-Br-cGMP (Fig. 8). The release observed with 300 μM of the analogue was 2.5 ± 0.20 times greater than that observed with 9 mM KCl alone. Cost considerations prevented testing of higher concentrations to determine the maximal effect of 8-Br-cGMP. The NO-donors appear to stimulate K^+-evoked release of [^{3}H]noradrenaline through the formation of cGMP since this release was partially inhibited by 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one and could be mimicked by 8-Br-cGMP.

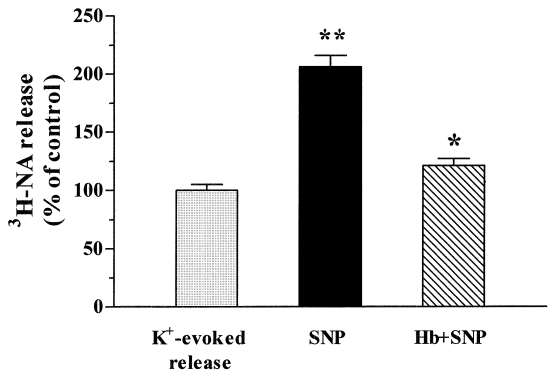

Fig. 7. Effect of the NO scavenger hemoglobin on enhanced K^+-evoked release of [^{3}H]noradrenaline elicited by 300 μM sodium nitroprusside. After 30 min equilibration, 20 μM hemoglobin was added to the perfusion medium. Eight minutes later the synaptosomes were stimulated with 9 mM KCl plus 300 μM sodium nitroprusside. Hemoglobin reduced the increment induced by sodium nitroprusside by 80%. Each column represents the mean ± S.E.M. of three experiments run in triplicate. $**\ P < 0.01$ versus K^+-evoked release; $*\ P < 0.01$ versus sodium nitroprusside treatment.

4. Discussion

Because of its gaseous nature, NO diffuses rapidly across the cell membrane, and it can thus transmit a broad-range signal that complements the point-to-point signals of other transmitters. The present study was prompted by the suggestion that NO produced at the postsynaptic level might act as a retrograde messenger to modify presynaptic release of other neurotransmitters (O'Dell et al., 1991). The use of isolated superfused synaptosomes allowed us to focus on the mechanisms that regulate neurotransmitter release with minimal interference by other structures of the central nervous system. Specific immunohistochemical studies have revealed fairly high concentrations of NO synthase in the cerebral cortex (Bredt et al., 1990). NO itself appears to play an important role in the functional development of the cortex (Vincent and Hope, 1992). Previous studies have demonstrated that NO is capable of stimulating the release of noradrenaline from the hippocampus (Lauth et al., 1995; Lonart and Johnson, 1995a,b; Satoh et al., 1996; Stout and Woodward, 1994). However other groups have found that NO exert an inhibitory effect on noradrenaline release (Seilicovich et al., 1995).

Both of the NO donors used in our study caused concentration-dependent increases in the basal and K^+-evoked release of [^{3}H]noradrenaline from synaptosomes. The differential potency of sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine observed, that probably re-

flect the amounts of NO that are liberated from these compounds, have also been reported by other authors (Ohkuma et al., 1995). The effect of hemoglobin (a known NO scavenger) on the enhanced release induced by both NO donors, together with the results of experiments using cyanide ions, strongly suggests that the enhanced release of the neurotransmitter is a result of the NO liberated by these compounds into the extracellular environment. Because of its size and charge, the hemoglobin molecule would not have been capable of neutralizing NO within the cell. The fact that the effects of the NO donors were not completely abolished by hemoglobin might be due to saturation of the latter or the spontaneous liberation of NO within the synaptosomes themselves.

Despite results obtained by Lonart and Johnson (1995b) which demonstrated that NO-generators induce a Ca^{2+}-dependent [^{3}H]noradrenaline release from hippocampal synaptopsomes mediated by reverse noradrenaline-transport, we have observed that the enhancement of basal release provoked by sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine proved to be Ca^{2+}-independent. Furthermore the results of the experiments with desipramine exclude the possibility that this effect is the result of an inversion of the carrier-mediated transport mechanism.

Although NO has been implicated in cytotoxic events in central nervous system related to anoxia (Oury et al., 1992), the release of [^{3}H]noradrenaline that we observed does not appear to be a manifestation of NO toxicity. The mechanisms underlying the cytotoxic effects of NO (inhibition of mitochondrial respiration, protein synthesis of a range of enzymes, destruction of ribonucleic reductase, etc.) are, for the most part, events that become manifest later in the cell cycle, and it is unlikely that they were responsible for the almost immediate release of the transmitter produced by the NO donors. Moreover, removal of the NO donor from the perfusion medium was followed by a gradual decrease in release to prestimulation values, and 35 min later, the synaptosomes were capable of responding normally to the depolarizing effects of K^+ ions.

Although many of the effects of NO are known to be mediated by cGMP, this does not seem to be the case for the NO-donor-stimulated release of synaptosomes under basal conditions. In fact, the permeable cGMP analogue 8-Br-cGMP had no effect on the release of [^{3}H]noradrenaline, and the release induced by sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine was not at all diminished by the soluble guanylate-cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one. Meffert et al. (1994) have shown that NO can produce Ca^{2+}- and guanylate cyclase-independent stimulation of dopamine release from hippocampal synaptosomes. These investigators suggest that this release is a result of activation of vesicular proteins caused by NO binding to thiol groups in the latter molecules (Meffert et al., 1996).

The increases produced by the NO-donors on basal and K^+- stimulated release of the [^{3}H]noradrenaline were very similar and both were obtained with high doses of the NO-donors; furthermore the increase induced by the NO-donors on basal release become apparent only after 9 min and gradually returned to prestimulation value. Conversely the effect of the NO-donors on release of the transmitter during K^+-stimulated depolarization appeared immediately after the application of the stimulus and was rapidly reverted.

But the more relevant difference was that the enhancement of K^+-evoked release induced by the NO-donors appear to be mediated by the formation of cGMP. In fact, the effects of sodium nitroprusside and *S*-nitroso-*N*-acetylpenicillamine were significantly reduced by the soluble guanylate cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one, and reproduced by the cGMP analogue, 8-Br-cGMP. Since the enhanced release was only partially abolished by 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one, it is possible that direct nitrosylation of the vesicular proteins also contributed to the effects we observed. cGMP has been shown to increase the release of other neurotransmitters from presynaptic terminals, and it is believed to function as the common pathway of retrograde messengers (Arancio et al., 1995; Zhuo et al., 1994a,b). cGMP and cGMP-dependent protein kinase are both capable of modulating membrane potential and ion channels (Butt et al., 1993). A cGMP-mediated enhancement of Ca^{2+} channel currents in rat sympathetic neurons has also been described (Chen and Schofield, 1995). It is therefore possible that NO's enhancement of noradrenaline release during depolarization is caused by cGMP-mediated changes in activated voltage-dependent Ca^{2+} channels. It would be interesting to verify this hypothesis with additional studies to identify the specific channels that are modulated by cGMP.

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