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# Inhibition of high $K^+$-evoked γ-aminobutyric acid release by sodium nitroprusside in rat hippocampus

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**Abstract**

To clarify whether nitric oxide (NO) modifies high $K^+$-evoked γ-aminobutyric acid (GABA) release, we examined the effects of sodium nitroprusside, an NO donor; diethyldithiocarbamate, an NO trapper; dithiothreitol, a superoxide radical scavenger; and 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one, a specific guanylyl cyclase inhibitor, on high (100 mM) $K^+$-evoked GABA release from rat hippocampus in vivo using microdialysis. Perfusion with 0.5 or 5 mM sodium nitroprusside significantly reduced high $K^+$-evoked GABA release. Co-perfusion with 0.5 mM sodium nitroprusside and 5 mM diethyldithiocarbamate or 0.5 mM 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one significantly enhanced high $K^+$-evoked GABA release. Co-perfusion with 0.5 mM sodium nitroprusside and 1 mM dithiothreitol tended to increase it. These results demonstrate that sodium nitroprusside reduces high $K^+$-evoked GABA release both via an NO/cyclic GMP-dependent pathway and via an NO-dependent, but cyclic GMP-independent, pathway in rat hippocampus in vivo.


*Keywords:* GABA (γ-Aminobutyric acid); Nitric oxide (NO); Sodium nitroprusside; Hippocampus; Microdialysis

## 1. Introduction

Nitric oxide (NO) is a molecular mediator that has been implicated in many physiological and pathological processes (see Bredt and Snyder, 1994; Szabo, 1996 for review). The NO synthase inhibitor, $N^G$-nitro-L-arginine methyl ester (L-NAME), at a low concentration (100 μM) enhanced *N*-methyl-D-aspartate (NMDA)-evoked γ-aminobutyric acid (GABA) release from the hippocampus of freely moving rats in vivo, although at high (0.2–1 mM) concentrations, L-NAME decreased or abolished the evoked GABA release (Getting et al., 1996). Sequeira et al. (1998) showed that an NO donor, hydroxylamine, reduced $Ca^{2+}$-dependent, high $K^+$-evoked GABA release in hippocampal synaptosomes. Schaffhauser et al. (1998) provided evidence that at least two metabotropic glutamate (mGlu) receptor subtypes, one with a group II- and one with a group III-like pharmacology, are involved in the mGlu receptor-mediated inhibition of KCl-induced [$^3$H]GABA release in rat cortical cultures. Raiteri et al. (2002) showed, using superfused rat cerebrocortex synaptosomes, that extracellular $K^+$ concentrations occurring in the central nervous system under certain pathological conditions provoke GABA release by mechanisms different from classical exocytosis, and that these include carrier-mediated release and internal $Ca^{2+}$-dependent exocytosis, the latter involving mitochondrial $Ca^{2+}$ in what seems to be a primary role.

However, to our knowledge, there are no in vivo studies of the involvement of NO in high $K^+$-evoked GABA release. Therefore, we examined the effects of an NO donor, sodium nitroprusside; an NO trapper, diethyldithiocarbamate; a superoxide radical scavenger, dithiothreitol; and 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one, a specific guanylyl cyclase inhibitor, on high $K^+$-evoked GABA release from rat hippocampus in vivo using microdialysis.

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## 2. Materials and methods

### 2.1. Animals and surgery

The experimental procedures have been described in detail in our previous papers (Hada et al., 2000; Kaku et al., 2001). The Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan) was strictly followed. In brief, male adult Wistar rats weighing 280–320 g were anesthetized with urethane (1.2 g/kg, i.p.) during experiments. We stereotaxically implanted a microdialysis probe with a 2-mm-active membrane (CMA/10, CMA/Microdialysis, Sweden) into the dorsal hippocampus to apply drugs and to collect dialysates. The basal perfusion medium was an artificial cerebrospinal fluid (ACSF) (composition in mM: NaCl, 132.8; KCl, 3.0; $CaCl_2$, 2.0; $MgCl_2$, 0.7; $NaHCO_3$, 24.6; urea, 6.7; glucose, 3.7). The perfusion flow rate was 2.0 μl/min and controlled by a microinjection pump (CMA/100, CMA/Microdialysis, Sweden).

### 2.2. Experimental protocol

Six groups of rats were dialysed through probes with a 2-mm-active membrane length. Four rats served as a control and received 100 mM $K^+$ alone for 30 min (control group). Five rats received 0.05 mM sodium nitroprusside + 100 mM $K^+$ for 30 min (0.05 mM sodium nitroprusside group). Four rats received 0.5 mM sodium nitroprusside + 100 mM $K^+$ for 30 min (0.5 mM sodium nitroprusside group). Six rats received 5 mM sodium nitroprusside + 100 mM $K^+$ for 30 min (5 mM sodium nitroprusside group). Four rats received 0.5 mM sodium nitroprusside + 5 mM diethyldithiocarbamate + 100 mM $K^+$ for 30 min (diethyldithiocarbamate group). Five rats received 0.5 mM sodium nitroprusside + 1 mM dithiothreitol + 100 mM $K^+$ for 30 min (dithiothreitol group). Three rats received 0.5 mM sodium nitroprusside + 0.5 mM 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one + 100 mM $K^+$ for 30 min (1*H*-(1,2,4)oxadiazole(4,3-a) quinoxalin-1-one group). Sodium nitroprusside, diethyldithiocarbamate, dithiothreitol or 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one was applied 30 min before perfusion with 100 mM $K^+$ for 30 min and thus the total perfusion time was 60 min.

Dialysates were collected every 10 min. GABA content was determined after precolumn derivatization with *o*-phthaldialdehyde by high-performance liquid chromatography (HPLC) using a fluorescence detector (CMA/100, CMA/Microdialysis, Sweden). A capillary column (BAS, C-18, 5 μm, monomeric 1.0 ϕ × 100 mm) was used for GABA analysis. The mobile phase was phosphate buffer (pH 6.0) with 0.1 mM EDTA-2Na, 10% acetonitrile and 3% tetrahydrofuran and was pumped at a flow rate of 60 μl/min.

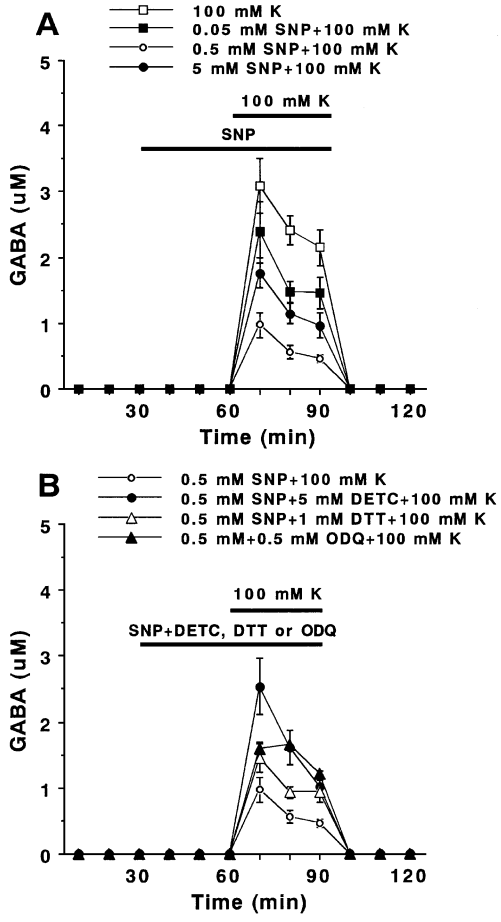


Fig. 1. Effect of sodium nitroprusside on high $K^+$-evoked GABA release. (A) Time course of GABA release evoked by perfusion with 100 mM $K^+$ alone (□), 0.05 mM sodium nitroprusside (SNP) + 100 mM $K^+$ (■), 0.5 mM sodium nitroprusside (SNP) + 100 mM $K^+$ (○) or 5 mM sodium nitroprusside (SNP) + 100 mM $K^+$ (●). Each point with a vertical bar represents the mean ± S.E.M. amount of GABA released in four to six experiments. ANOVA of data for high $K^+$-evoked GABA release revealed that the main effects of group and time, and an interaction between group and time, were significant ($F(3/15) = 11.289$, $P = 0.0004$; $F(11/165) = 151.665$, $P < 0.0001$; $F(33/165) = 8.288$, $P < 0.0001$, respectively). Fisher's post hoc tests revealed significant differences between the high $K^+$ and the 0.05, 0.5 and 5 mM sodium nitroprusside groups ($P = 0.0299$; $P < 0.0001$; $P < 0.001$, respectively). (B) Time course of GABA release evoked by perfusion with 0.5 mM sodium nitroprusside (SNP) + 100 mM $K^+$ (○), 0.5 mM sodium nitroprusside (SNP) + 5 mM diethyldithiocarbamate (DETC) + 100 mM $K^+$ (●), 0.5 mM sodium nitroprusside (SNP) + 1 mM dithiothreitol (DTT) + 100 mM $K^+$ (△) or 0.5 mM sodium nitroprusside (SNP) + 0.5 mM 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one (ODQ) + 100 mM $K^+$ (▲). Each point with a vertical bar represents the mean ± S.E.M. amount of GABA released in four or five experiments. ANOVA of data for high $K^+$-evoked GABA release revealed that the main effects of group and time, and an interaction between group and time, were significant ($F(2/10) = 8.792$, $P = 0.0063$; $F(11/110) = 97.578$, $P < 0.0001$; $F(22/110) = 7.182$, $P < 0.0001$, respectively). Fisher's post hoc tests revealed significant differences between the 0.5 mM sodium nitroprusside and the diethyldithiocarbamate groups and between the diethyldithiocarbamate and the dithiothreitol groups ($P = 0.0019$ and $P = 0.0001$, respectively).

### 2.3. Drugs

Sodium nitroprusside, diethyldithiocarbamate, dithiothreitol and 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one were purchased from Sigma (St. Louis, MO) and dissolved in ACSF.

### 2.4. Statistical analysis

The data are expressed as means ± S.E.M. To determine the difference between experimental groups and the time course of GABA release, statistical analysis was performed by two-way analysis of variance (ANOVA) with repeated measures and post hoc tests (Fisher's protected least significant difference). $P$ values $<0.05$ were considered to be significant.

## 3. Results

By means of the HPLC system used in this study, we could not detect basal concentrations of GABA release in any group. Fig. 1 shows the time course for high $K^+$-evoked GABA release in the high $K^+$ alone (control group), and 0.05, 0.5 and 5 mM sodium nitroprusside, diethyldithiocarbamate, dithiothreitol and 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one groups. In all groups, perfusion with 100 mM $K^+$ increased GABA release with a similar pattern. The GABA levels abruptly reached maximum values immediately (10 min) after the onset of perfusion with 100 mM $K^+$. Perfusion with high $K^+$ alone increased GABA release to a maximum value of 3.1 ± 0.4 μM ($n=4$). Perfusion with 0.05, 0.5 and 5 mM sodium nitroprusside reduced the high $K^+$-evoked GABA release to a maximum of 2.4 ± 0.5 ($n=5$), 1.0 ± 0.2 ($n=4$) and 1.8 ± 0.2 μM ($n=6$), respectively (Fig. 1A). Co-perfusion with 0.5 mM sodium nitroprusside and 5 mM diethyldithiocarbamate significantly increased the high $K^+$-evoked GABA release to a maximum value of 2.5 ± 0.5 μM ($n=4$) (Fig. 1B). Co-perfusion with 0.5 mM sodium nitroprusside and 1 mM dithiothreitol significantly increased the high $K^+$-evoked GABA release to a maximum value of 1.5 ± 0.2 μM ($n=5$). Co-perfusion with 0.5 mM sodium nitroprusside and 0.5 mM 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one significantly increased the high $K^+$-evoked GABA release to a maximum value of 1.6 ± 0.1 μM ($n=3$).

## 4. Discussion

The principal findings of the present in vivo study were as follows: (1) the NO donor, sodium nitroprusside, reduced high $K^+$-evoked GABA release from rat hippocampus; (2) this inhibition was partly reversed by the NO trapper, diethyldithiocarbamate, and the specific guanylyl cyclase inhibitor, 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one; and (3) the superoxide radical scavenger, dithiothreitol, did not modulate the evoked GABA release. These results indicate that the sodium nitroprusside-induced inhibition of high $K^+$-evoked GABA release is due to NO.

In the present study, sodium nitroprusside reduced high $K^+$-evoked GABA release. Sodium nitroprusside at 5 mM was less active than at 0.5 mM. Because 5 mM of sodium nitroprusside could be more toxic than 0.5 mM, excess NO produced by sodium nitroprusside might inhibit GABA uptake and cause GABA to leak from GABAergic neurons. Consequently, 5 mM of sodium nitroprusside would decrease the inhibition of high $K^+$-evoked GABA release. The present in vivo finding that sodium nitroprusside reduced high $K^+$-evoked GABA release is in accordance with the in vitro finding of Sequeira et al. (1998), showing that hydroxylamine, an NO donor, reduced high $K^+$-evoked GABA release from rat hippocampal synaptosomes. Bie and Zhao (2001) have demonstrated that sodium nitroprusside reversibly decreases the inward current evoked by GABA via the $GABA_A$ receptor in isolated rat dorsal root ganglion neurons via a cyclic GMP-dependent protein kinase pathway. However, our present result contrasts with the finding of Ientile et al. (1997) that NO (spermine/NO, a slow spontaneous releaser of this nitrogen radical) increases NMDA-evoked [$^3$H]GABA release from chick embryo retina cell cultures via an NO/cyclic GMP-dependent pathway.

The sodium nitroprusside-induced inhibition of high $K^+$-evoked GABA release observed in this study would be caused by NO, because the inhibition was partly reversed by the NO trapper, diethyldithiocarbamate. However, the reasons why diethyldithiocarbamate did not reverse it completely are not clear at present.

The inhibitory effect of sodium nitroprusside on the high $K^+$-evoked GABA release may be caused by a presynaptic action. This result is supported by the following studies. NO and NO-releasing agents blocked the NMDA-, kainate- and $K^+$-induced increase of $[Ca^{2+}]_i$ in cultured rat forebrain neurons (Hoyt et al., 1992). Tanaka et al. (1993) showed that L-$N^G$-nitroarginine, an NO synthase inhibitor, augmented the NMDA- and kainate-induced increase in the intracellular $Ca^{2+}$ concentration in rat hippocampal neurons, an effect that was blocked by L-arginine, a substrate for NO synthase. High $K^+$-induced increases in both $Ca^{2+}$ uptake and contraction were inhibited in the presence of 10 μM sodium nitroprusside in the isolated rat aortic smooth muscle (Karaki et al., 1988). Moreover, it was shown that sodium nitroprusside reduced GABA currents in hooded rat-cultured retinal amacrine cells (Wexler et al., 1998) and the $Cl^-$ current elicited by GABA in rat cerebellar granule cells (Zaari et al., 1994).

As for the mechanism(s) involved in the inhibition of the high $K^+$-evoked GABA release by NO, exogenous NO produced by the NO donor, sodium nitroprusside, increases the cyclic GMP levels due to activation of the

soluble gaunylyl cyclase. Then the increase in cyclic GMP decreases $Ca^{2+}$ influx (Desole et al., 1994), resulting in a reduction in GABA release. This explanation is supported by the present finding that the soluble gaunylyl cyclase inhibitor, 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one, partly reversed the inhibitory effect of sodium nitroprusside on the high $K^+$-evoked GABA release. Since NO enhances adenosine release from rat hippocampus in vitro (Fallahi et al., 1996; Rosenberg et al., 2000), the sodium nitroprusside-induced inhibition could be explained by the released adenosine acting through adenosine $A_1$ receptors on GABAergic terminals to reduce GABA release. Indeed, this is supported by our previous report (Hada et al., 1997) showing that adenosine $A_1$ receptor agonist inhibits high $K^+$-evoked GABA release from rat hippocampus in vivo. The NO-induced adenosine release may not include a cyclic GMP-dependent pathway, because 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one and cyclic GMP analogs had no effect on adenosine accumulation in rat forebrain neurons in culture (Rosenberg et al., 2000). Therefore, we consider at present that sodium nitroprusside inhibited high $K^+$-evoked GABA release not only via an NO/cyclic GMP-dependent pathway but also via a cyclic GMP-independent pathway.

The present finding that the high $K^+$-evoked GABA release decreased with time (Fig. 1) could be explained as follows. High $K^+$ increases the release of aspartate and glutamate. The increase in excitatory amino acids may activate kainate receptors on GABAergic terminals, resulting in a reduction in GABA release. Indeed, Cunha et al. (1997) found that kainate inhibited [$^3$H]GABA release from rat hippocampal synaptosomes. Kainate reversibly depressed monosynaptically activated inhibitory post-synaptic potentials in rat hippocampal CA3 pyramidal cells (Clarke et al., 1997). Moreover, Rodriguez-Moreno and Lerma (1998) reported that activation of kainate receptors decreased the amplitude of evoked inhibitory post-synaptic currents of rat hippocampal CA1 pyramidal cells, possibly through the metabotropic function of kainate receptors.

We have recently observed that perfusion with 0.5 mM sodium nitroprusside alone or 0.5 mM sodium nitroprusside + high $K^+$ always induces seizures (sodium nitroprusside-induced seizures) (Hada et al., 2000; Kaku et al., 2001). Diethyldithiocarbamate, the NO trapper, inhibits sodium nitroprusside-induced seizures. This could be explained as follows: the increase in extracellular GABA levels produced by diethyldithiocarbamate hyperpolarizes hippocampal neurons, resulting in abolition of the sodium nitroprusside-induced seizures.

In conclusion, the present study demonstrates, for the first time, that an NO donor, sodium nitroprusside, reduces high $K^+$-evoked GABA release both via an NO/cyclic GMP-dependent pathway and via a cyclic GMP-independent pathway from rat hippocampus in vivo. Sodium nitroprusside reduced high $K^+$-evoked GABA release and diethyldithiocarbamate and 1*H*-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one antagonized partly the sodium nitroprusside-induced inhibition.

## References


Bie, B.-H., Zhao, Z.-Q., 2001. Nitric oxide inhibits GABA-evoked current in dorsal root ganglion neuron via PKG-dependent pathway. Brain Res. Bull. 55, 335–339.

Bredt, D., Snyder, S.A., 1994. Nitric oxide: a physiologic messenger molecule. Ann. Rev. Biochem. 63, 175–195.

Clarke, V.R.J., Ballyk, B.A., Hoo, K.H., Mandelzys, A., Pellizzari, A., Bath, C.P., Thomas, J., Sharpe, E.F., Davies, C.H., Ornstein, P.L., Schoepp, D.D., Kamboj, R.K., Collingridge, G.L., Lodge, D., Bleakman, D., 1997. A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. Nature 389, 599–603.

Cunha, R.A., Constantino, M.D., Ribeiro, J.A., 1997. Inhibition of [$^3$H]γ-aminobutyric acid release by kainate receptor activation in rat hippocampal synaptosomes. Eur. J. Pharmacol. 323, 167–172.

Desole, M.S., Kim, W.-K., Rabin, R.A., Laychock, S.G., 1994. Nitric oxide reduces depolarization-induced calcium influx in PC12 cells by a cyclic GMP-mediated mechanism. Neuropharmacology 33, 193–198.

Fallahi, N., Broad, R., Jin, S., Fredholm, B.B., 1996. Release of adenosine from rat hippocampal slices by nitric oxide donors. J. Neurochem. 67, 186–193.

Getting, S.J., Segieth, J., Ahmad, S., Biggs, C.S., Whitton, P.S., 1996. Biphasic modulation of GABA release by nitric oxide in the hippocampus of freely moving rats in vivo. Brain Res. 717, 196–199.

Hada, J., Kaku, T., Morimoto, K., Hayashi, Y., Nagai, K., 1997. PIA, an adenosine $A_1$ receptor agonist, reduces high $K^+$-evoked GABA release from in vivo rat hippocampus. In: Okada, Y. (Ed.), The Role of Adenosine in the Nervous System. Elsevier, Amsterdam, pp. 151–156.

Hada, J., Kaku, T., Jiang, M.-H., Morimoto, K., Hayashi, Y., Nagai, K., 2000. Sodium nitroprusside-induced seizure and taurine release from rat hippocampus. Amino Acids 19, 547–559.

Hoyt, K.R., Tang, L.-H., Aizenman, E., Reynolds, I.J., 1992. Nitric oxide modulates NMDA-induced increases in intracellular $Ca^{2+}$ in cultured rat forebrain neurons. Brain Res. 592, 310–316.

Ientile, R., Pedale, S., Picciurro, V., Macaione, V., Fabiano, C., Macaione, S., 1997. Nitric oxide mediates NMDA-evoked [$^3$H]GABA release from chick retina cells. FEBS Lett. 417, 345–348.

Kaku, T., Jiang, M.-H., Hada, J., Morimoto, K., Hayashi, Y., 2001. Sodium nitroprusside-induced seizures and adenosine release in rat hippocampus. Eur. J. Pharmacol. 413, 199–205.

Karaki, H., Sato, K., Murakami, K., 1988. Effects of sodium nitroprusside on cytosolic calcium level in vascular smooth muscle. Eur. J. Pharmacol. 156, 259–266.

Raiteri, L., Stigliani, S., Zedda, L., Raiteri, M., Bonanno, G., 2002. Multiple mechanisms of transmitter release evoked by 'pathologically' elevated extracellular [$K^+$]: involvement of transporter reversal and mitochondrial calcium. J. Neurochem. 80, 706–714.

Rodriguez-Moreno, A., Lerma, J., 1998. Kainate receptor modulation of GABA release involves a metabotropic function. Neuron 20, 1211–1218.

Rosenberg, P.A., Li, Y., Le, M., Zhang, Y., 2000. Nitric oxide-stimulated increase in extracellular adenosine accumulation in rat forebrain neurons in culture is associated with ATP hydrolysis and inhibition of adenosine kinase activity. J. Neurosci. 20, 6294–6301.

Schaffhauser, H., Knoflach, F., Pink, J.R., Bleuel, Z., Cartmell, J., Goepfert, F., Kemp, J.A., Richards, J.G., Adam, G., Mutel, V., 1998. Multiple pathways for regulation of the KCl-induced [$^3$H]-GABA release by metabotropic glutamate receptors, in primary rat cortical cultures. Brain Res. 782, 91–104.

Sequeira, S.M., Duarte, C.B., Carvalho, A.P., Carvalho, C.M., 1998. Nitric oxide differentially affects the exocytotic and the carrier-mediated re-

lease of [$^3$H]γ-aminobutyric acid in rat hippocampal synaptosomes. Mol. Brain Res. 55, 337–340.

Szabo, C., 1996. Physiological and pathophysiological roles of nitric oxide in the central nervous system. Brain Res. Bull. 41, 131–141.

Tanaka, T., Saito, H., Matsuki, N., 1993. Endogenous nitric oxide inhibits NMDA- and kainate-responses by a negative feedback system in rat hippocampal neurons. Brain Res. 631, 72–76.

Wexler, E.M., Stanton, P.K., Nawy, S., 1998. Nitric oxide depresses $GABA_A$ receptor function via coactivation of cGMP-dependent kinase and phosphodiesterase. J. Neurosci. 18, 2342–2349.

Zaari, I., Bucossi, G., Cupello, A., Rapallino, M.V., Robello, M., 1994. Modulation by nitric oxide of rat brain $GABA_A$ receptors. Neurosci. Lett. 180, 239–242.