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# Extracellular cGMP in the Hippocampus of Freely Moving Rats as an Index of Nitric Oxide (NO) Synthase Activity

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**The nitric oxide (NO) synthase/cGMP pathway has been studied *in vivo* in the adult rat hippocampus by monitoring the levels of extracellular cGMP during microdialysis in conscious unrestrained animals. The basal cGMP efflux was concentration-dependently reduced upon local infusion of the NO synthase inhibitor $N^G$-nitro-L-arginine (NARG; 10 μM to 1 mM). The NO donors hydroxylamine and *S*-nitroso-*N*-penicillamine, perfused through the dialysis probe at 1 mM, increased by about 200% the extracellular levels of cGMP. The glutamate receptor agonist NMDA (125–500 μM) produced concentration-dependent cGMP responses that were abolished by the selective receptor antagonist D-2-amino-5-phosphonovaleric acid or by NARG. Local perfusion of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 1 mM) produced a steady eightfold increase of extracellular cGMP levels. The effect of IBMX was highly sensitive to NARG. The inhibition by NARG of the IBMX-induced cGMP response was reversed when the NO synthase substrate L-arginine was administered. It is concluded that cGMP collected during *in vivo* microdialysis reflects NO synthase activity in the rat hippocampus. The technique may be utilized to investigate the pathophysiology and the pharmacology of the NO/cGMP pathway in the hippocampus of living animals.**

***[Key words: nitric oxide synthase, in vivo microdialysis, hippocampus, nitric oxide, cGMP, phosphodiesterase, nitric oxide donors]***

Nitric oxide (NO) is being recognized as an important messenger molecule in an increasing number of mammalian tissues (Moncada et al., 1989). NO is formed from the amino acid L-arginine, under the catalysis of NO synthase (Knowles et al., 1989; Bredt and Snyder, 1990). In immunohistochemical studies NO synthase was observed in neurons throughout the brain and the discrete pattern of its localization suggests particular functions for NO as a signaling molecule in synaptic transmission (Garthwaite, 1991; Snyder and Bredt, 1991).

NO is a potent activator of heme-containing soluble guanylate cyclase and causes cGMP formation in target cells (for reviews, see Waldman and Murad, 1987; Garthwaite, 1991; Snyder and Bredt, 1991). Enhancement of NO production responsible for cGMP elevation has been described in cerebellar slices of immature and adult rats in response to NMDA receptor activation (Garthwaite et al., 1988; Southam et al., 1991). The NO/cGMP pathway has been described in hippocampal slices of immature rats, and preliminary results suggest that cGMP accumulation linked to NMDA receptor activation also occurs in slices of adult rat hippocampus, although the cGMP response observed was much less pronounced than in slices from the adult cerebellum (East and Garthwaite, 1991).

The hippocampal formation is considered to play a central role in learning and memory processes. Activation of NMDA receptors in this area is essential to the development of long-term potentiation (LTP), a mechanism thought to represent a mnemonic device (see Bliss and Collingridge, 1993). Recent reports suggest that NO may act as a retrograde messenger in LTP, carrying information from postsynaptic to presynaptic elements (O'Dell et al., 1991; Schuman and Madison, 1991). Similarly, the NMDA receptor → NO → cGMP pathway has been implicated in long-term depression (Shibuki and Okada, 1991), a possible cellular mechanism for cerebellar motor learning (Ito, 1989).

A number of pharmacological tools are now available to investigate the NO/cGMP pathway, including inhibitors of NO synthesis, NO scavengers, NO generators, and inhibitors of the cyclic nucleotide phosphodiesterases. The availability of selective drugs and the advancement of *in vivo* sampling techniques represented by intracerebral microdialysis (Ungerstedt, 1984) might allow study of the NO/cGMP system and its modulations in selected brain areas of the living animal. This work describes the changes of extracellular cGMP caused, in the rat hippocampus subjected to *in vivo* microdialysis, by administration of drugs expected to affect the NO/cGMP pathway.

## Materials and Methods

*Animals.* Male Sprague–Dawley rats (CD-COBS, Charles River, Calco, Italy) weighing 250–300 gm were used. They were housed at constant room temperature (22 ± 1°C) and relative humidity (50%) under a regular light/dark schedule (light 7 A.M. to 7 P.M.). Food and water were freely available.

*Dialysis procedure.* Rats were anesthetized with 3 ml/kg Equithesin (pentobarbital, 9.6 gm/liter; chloral hydrate, 42.4 gm/liter; $MgSO_4$, 21.2 gm/liter; propylene glycol, 396 gm/liter; ethanol, 100 gm/liter) and placed on a stereotaxic apparatus (David Kopf Instruments). A dialysis probe was transversely positioned in the two dorsal hippocampi of the rat. Stereotaxic coordinates were AP = +3.8, H = +6.5 from the interaural line according to the Paxinos and Watson atlas (1986). A short piece of dialysis fiber made of a copolymer of acrylonitrile sodium methallyl sulfonate (AN 69HF, Hospal SpA, Bologna, Italy; 0.3 mm o.d. with more than 40,000 Da cutoff) was covered with epoxy glue to confine dialysis to the area of interest (8 mm glue-free zone for the two hippocampi). The rat's skull was exposed and two holes were made on the lateral surface at the level of dorsal hippocampus. One dialysis probe,

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held straight by a tungsten wire inside, was inserted transversely into the brain so that the glue-free zone was exactly located in the target area. The tungsten wire was withdrawn and stainless steel cannulas (22 gauge diameter, about 15 mm long) were glued to the ends of the fiber. These ends were bent up and fixed vertically to the skull using dental cement and modified Eppendorf tips. Finally, the skin was sutured and the rats were allowed to recover from anesthesia for 24 hr before the beginning of experiment.

On the day of the experiment the rat was placed in a Perspex cage and the probe was perfused with an artificial cerebrospinal fluid (CSF) containing (in mM) 145 NaCl, 3 KCl, 1.26 $CaCl_2$, 1 $MgCl_2$, in distilled water. The solution was buffered at pH 7.4 with a 2 mM sodium phosphate buffer.

The fibers were perfused at a constant flow rate of 5 μl/min with a CMA/100 microinjection pump (Carnergie Medicin, Stockholm, Sweden), and every 20 min samples of perfusate were collected in minivials and assayed for their cGMP content. cGMP was determined using a commercially available radioimmunoassay kit (Amersham dual range, Amersham Radiochemical Centre, Buckinghamshire, U.K.). The sensitivity of the assay was about 1.0 fmol/sample. The percentage of *in vitro* recovery for cGMP was 40 ± 1.2 ($n$ = 3) in our condition of perfusion (flow rate, 5 μl/min; 100 μl/sample). The correct placement of the dialysis probes in the hippocampi was verified by histological examination of fiber tracts.

*Statistics and expression of results.* The average of the three 20 min samples collected just before drug treatment was considered as basal value and defined as 100%. The values given are expressed as percentages of basal. Data on Figure 3 are expressed as fmol/100 μl (data not corrected for recovery). The effects between control animals (artificial CSF alone) and treated animals (artificial CSF + drugs) were analyzed by a single two-way ANOVA with repeated measures over time. When significant effects were found, post hoc between comparisons were carried out with Tukey's test.

*Materials.* L-Arginine, 3-isobutyl-1-methylxanthine (IBMX), and $N^G$-nitro-L-arginine (NARG) were of the purest grade available (Sigma Chemical Co., St. Louis, MO). NMDA and D-2-amino-5-phosphonovaleric acid (D-AP5) were purchased from Tocris Neuramin (Bristol, U.K.). *S*-nitroso-*N*-penicillamine (SNAP) was obtained from Cookson Chemicals Ltd. (Southampton, U.K.). Hydroxylamine was obtained from Merck (Darmstadt, Germany). IBMX was dissolved in NaOH (0.1 M) to a concentration of 100 mM; subsequent dilutions were made in artificial CSF. All the other drugs were dissolved in artificial CSF.

The experimental procedures *in vivo* were approved by the Ethical Committee of the Institute of Pharmacology and Pharmacognosy according to the European legislation on the use and care of laboratory animals (CEE 86/605).

## Results

*Effects of NO synthase inhibition on basal cGMP efflux.* The average basal levels of cGMP in the hippocampal dialysate did not differ significantly between experiments (18.9 ± 1.8 fmol/100 μl; means ± SEM of three consecutive 20 min samples obtained from 30 animals; data not corrected for *in vitro* recovery). The basal efflux was stable with time and detectable for at least 140 min. When $Ca^{2+}$ ions were omitted from the perfusion medium, the basal cGMP outflow was significantly decreased (data not shown).

When the cGMP levels in the dialysates were monitored during infusion with the NO synthase inhibitor NARG, the basal efflux of cGMP was reduced by the drug (10 μM to 1 mM) in a concentration-dependent manner. The maximal inhibitory effect (about 55%) was reached 40 min after infusion of NARG at 1 mM (Fig. 1).

*Effects of NO generators.* As shown in Figure 2*A*, hydroxylamine (1 mM) perfused through the probe for 80 min increased by approximately 200% the extracellular levels of cGMP. A quantitatively similar increase of the cGMP recovered in the dialysates was produced by infusing for 80 min 1 mM SNAP, another NO donor (Fig. 2*B*).

*Extracellular cGMP following infusion of NMDA.* The glutamate receptor agonist NMDA (125–500 μM), perfused through the dialysis probe for 20 min, produced a dose-related increase in the extracellular concentrations of cGMP (Figs. 3, 4). At the concentration of 500 μM the outflow of cGMP reached a maximum at the end of the infusion with NMDA (time = 80 min in Fig. 3) and rapidly returned to basal in the subsequent fraction. The cGMP levels returned to normal by time = 100 min also if the NMDA infusion was prolonged up to time = 140 min (not shown).

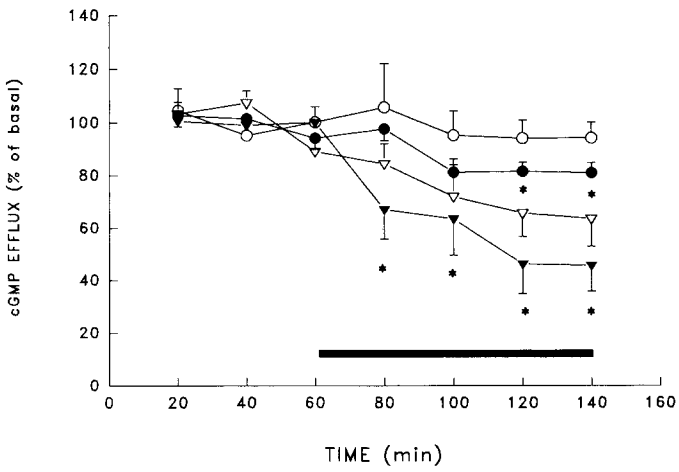


*Figure 1.* Effects of the NO synthase inhibitor NARG on basal extracellular levels of cGMP in the hippocampus of freely moving rats. Values are expressed as percentage of basal (±SEM; $n$ = 4–6 for each group). The *horizontal bar* indicates the time during which different concentrations of NARG were perfused through the dialysis probe. Basal absolute values of cGMP (34.4 ± 6.1 fmol/100 μl) did not differ significantly among the four sets of experiments and were grouped together. *, $P < 0.01$ versus controls and basal samples. For further details, see Materials and Methods. ○, controls; ●, NARG (10 μM); ▽, NARG (100 μM); ▼, NARG (1 mM).

As shown in Figure 4, the cGMP response caused by 500 μM NMDA was completely abolished when the selective NMDA receptor antagonist D-AP5 (500 μM) or the NO synthase inhibitor NARG (10 μM) was perfused for 60 min prior co-infusion with NMDA. D-AP5 (500 μM) had no significative influence, on its own, on basal cGMP levels, while NARG (100 μM) produced a significant decrease (Fig. 4).

*Extracellular cGMP during phosphodiesterase inhibition and effects of NO synthase modulation.* When the phosphodiesterase inhibitor IBMX was perfused through the dialysis probe at a concentration of 1 mM, a steady eightfold increase of extracellular cGMP levels was obtained (Fig. 5). The levels of cGMP in the dialysate, in presence of IBMX, were reduced by NARG (1 μM to 1 mM) in a concentration-dependent manner (Fig. 6). The maximal effect (about 70%) was reached 80 min after infusion of 1 mM NARG. Figure 7 shows that the NO synthase substrate L-arginine (1 mM) was able to increase by about 40% the extracellular concentration of cGMP during IBMX perfusion. L-Arginine (1 mM) consistently counteracted the inhibitory action of 100 μM NARG on the cGMP response.

*Behavioral observations during microdialysis.* The perfusion experiments were made the day after surgery to allow the animals to recover and to reduce the effects of anesthesia. In a group of operated but untreated animals, gross behavior and food and water intake appeared normal up to 6 d. No signs of pain or distress were evident throughout the experiments. Dur-

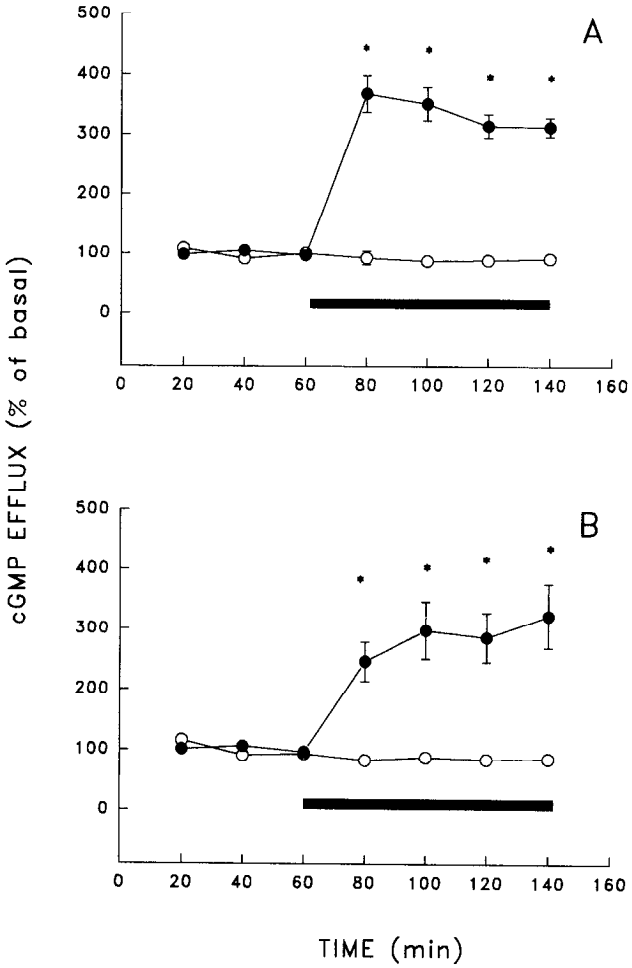


*Figure 2.* Effects of the NO generators hydroxylamine or SNAP on basal extracellular levels of cGMP in rat hippocampus of freely moving rats. Hydroxylamine or SNAP (1 mM) was perfused through the dialysis probe for 80 min as indicated by the *horizontal bar*. For other details, see Materials and Methods. Values are expressed as percentage of basal (±SEM; $n = 4$–6 for each group). Basal absolute values of cGMP (26.8 ± 5.5 fmol/100 μl) did not differ significantly between experiments and were grouped together. *A*: ○, controls; ●, hydroxylamine (1 mM). *B*: ○, controls; ●, SNAP (1 mM). *, $P < 0.01$ versus controls and basal samples.

ing infusion of artificial CSF, rats were resting or moving normally in the perfusion bowl.

When NMDA (125–250 μM; 20 min) was infused, mild behavioral excitation was observed, which included exploration of the cage, sniffing, and sporadic "wet-dog" shakes. During 20 min perfusion with 500 μM NMDA some rats (about 1 of 3) displayed a "wild running" behavior consisting in rapid running around the cage with frequent collisions with the walls. This "wild running" behavior had no apparent influence on the cGMP response. Higher concentration of NMDA perfused through the probe for 20 min produced marked seizures (head nodding, forelimb clonus, falling on side and rolling). For this reason concentrations of NMDA higher than 500 μM were not used. When the NMDA receptor antagonist D-AP5 was perfused for 40 min through the probe at the concentration of 500 μM, the behavior of the animals was unaffected. D-AP5 was able to prevent completely the behavioral activation induced by NMDA (500 μM). No episodes of "wild running" were observed during the administration of 500 μM NMDA in presence of D-AP5.

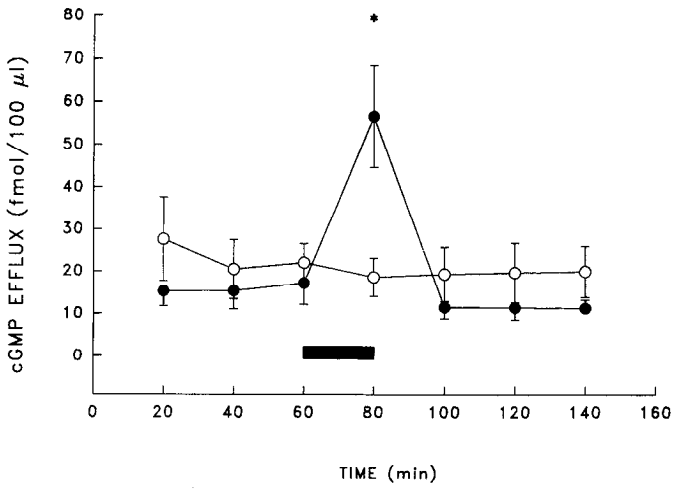


*Figure 3.* Effect of NMDA on basal extracellular levels of cGMP in rat hippocampus of freely moving rats. NMDA (500 μM) was perfused through the dialysis probe for 20 min as indicated by the *horizontal bar*. For other details, see Materials and Methods. Values are expressed as fmol/100 μl (±SEM; $n = 4$–6 for each group; data not corrected for *in vitro* recovery). ○, controls; ●, NMDA (500 μM). *, $P < 0.01$ versus controls and basal samples.

SNAP and hydroxylamine, at the concentration of 1 mM, did not produce any obvious change of the animal behavior. In particular, no seizures were observed by perfusing the NO donors at the concentration used in our experiments (1 mM). Only at 10 mM did hydroxylamine produce a mild behavioral activation consisting in sporadic "wet-dog" shakes, sniffing, rearing, and digging in the sawdust.

IBMX, infused locally at 1 mM throughout the experiment, did not modify the behavior of the rats. Similarly, the NO synthase inhibitor NARG (1–1000 μM) did not influence on its own the resting behavior of the animals, nor did it modify the behavioral activation induced by 500 μM NMDA. L-Arginine (1 mM) showed no significative alterations of the normal behavior of the rats.

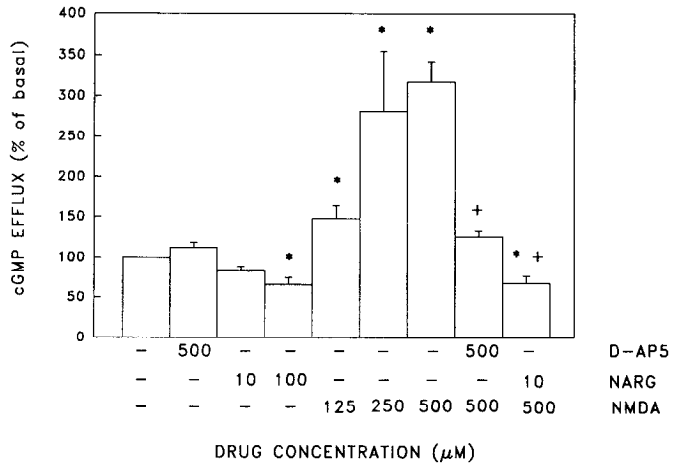


*Figure 4.* Effects of NMDA, NARG, and D-AP5 on the extracellular levels of cGMP in the hippocampus of freely moving rats. D-AP5 (500 μM) and NARG (10 or 100 μM) were infused for 60 min prior co-infusion (20 min) with NMDA (500 μM). Maximum responses are shown. The values are expressed as percentage of basal (±SEM; $n = 4$–6). *, $P < 0.01$ versus controls and basal samples. +, $P < 0.01$ versus 500 μM NMDA.

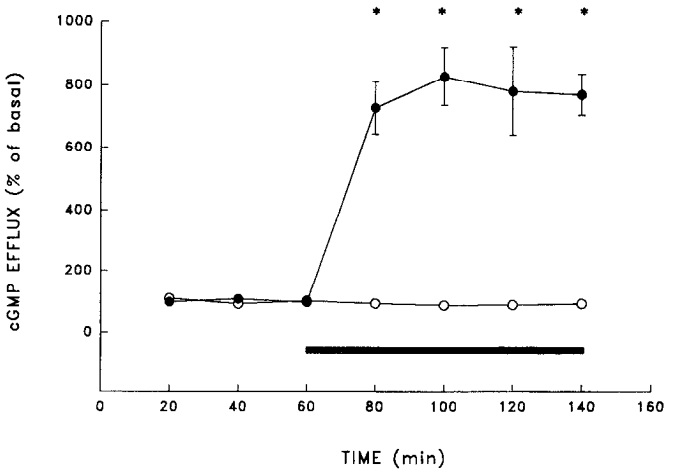


*Figure 5.* Effect of IBMX on basal extracellular levels of cGMP in rat hippocampus of freely moving rats. IBMX (1 mM) was perfused through the dialysis probe for 80 min as indicated by the *horizontal bar*. For other details, see Materials and Methods. Values are expressed as percentage of basal (±SEM; $n = 4$–6 for each group). Basal absolute values of cGMP (22.1 ± 3.7 fmol/100 μl) did not differ significantly between the two sets of experiments and were grouped together. ○, controls; ●, IBMX (1 mM). *, $P < 0.01$ versus controls and basal samples.

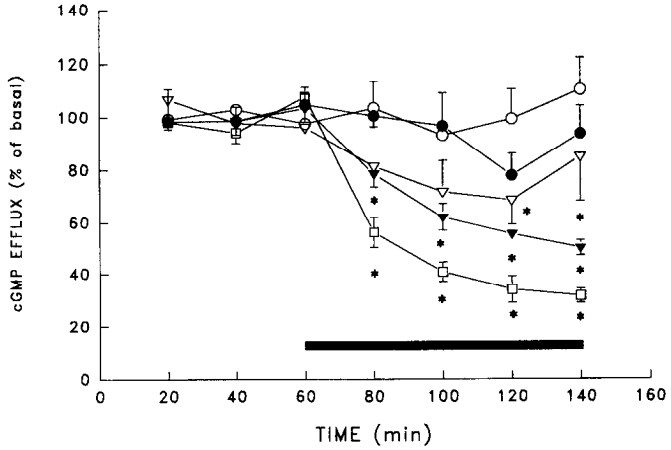


*Figure 6.* Effects of the NO synthase inhibitor NARG on extracellular levels of cGMP in the hippocampus of freely moving rats during IBMX perfusion. IBMX (1 mM) was perfused all throughout the experiment. The cGMP levels of the three 20 min fractions collected during IBMX perfusion but prior the administration of NARG were averaged and considered as "basal." Values are expressed as percentage of "basal" (±SEM; $n = 4$–6 for each group). The *horizontal bar* indicates the time during which different concentrations of NARG were perfused through the probe. "Basal" absolute values of cGMP (158.9 ± 13 fmol/100 μl) did not differ significantly among the five sets of experiments and were grouped together. ○, controls; ●, NARG (1 μM); ▽, NARG (10 μM); ▼, NARG (100 μM); □, NARG (1 mM). *, $P < 0.01$ versus controls and "basal" samples.

## Discussion

*Methodological aspects.* According to recent reports, the levels of extracellular cAMP measured during *in vivo* intracerebral microdialysis appear to reflect activation of adenylate cyclase mediated by brain aminergic receptors (Stone and John, 1990; Sijbesma et al., 1991).

In 1986, Tjörnhammar et al. found that a portion (10–15%) of the cGMP, produced intracellularly when cerebellar slices were depolarized with high $K^+$ or were exposed to glutamic acid, appeared in the extracellular space. The efflux of cGMP into the medium seemed to reflect the increase in the intracellular nucleotide. Moreover, the extracellular levels could be lowered by probenecid, a known blocker of anion transport in membranes, suggesting the existence of a carrier-mediated efflux for cGMP.

The pivotal role that NO seems to play as an intercellular messenger in the brain, particularly in the hippocampus where it has been proposed to be involved in various processes including LTP (O'Dell et al., 1991; Schuman and Madison, 1991), prompted us to investigate the possibility of monitoring, during *in vivo* hippocampal microdialysis, the extracellular levels of cGMP and to ascertain whether and to what extent the nucleotide present in the dialysates could reflect the activity of NO synthase, the enzyme that catalyzes the production of NO, a major activator of guanylyl cyclase.

In order to investigate the relations between extracellular cGMP in hippocampus dialysates obtained from freely moving rats and the activity of NO synthase, a number of pharmacological manipulations expected to affect the NO synthase → NO → guanylyl cyclase → cGMP pathway were applied.

*NO synthase inhibition.* A number of drugs have been used as selective inhibitors of the cGMP-linked NO synthase. One of the most effective, NARG, was employed in our experiments. As shown in Figure 1, NARG concentration-dependently inhibited the cGMP levels present in the dialysates under basal conditions. The inhibition by NARG was counteracted by the NO synthase substrate L-arginine (data not shown, but see Fig. 7), a finding consistent with the idea that NARG acts as a competitive inhibitor of the enzyme.

High concentrations of NARG (1 mM) were unable to abolish completely the basal outflow of cGMP, suggesting that a portion of the nucleotide recovered originates independently of an NARG-sensitive NO synthase. It should be noted that the brain NO synthase is $Ca^{2+}$ dependent and that the basal outflow of cGMP was only in part sensitive to $Ca^{2+}$ ions. The NARG-insensitive portion of cGMP could be generated by NO produced by NO synthase isoforms (Lambert et al., 1991; Moncada

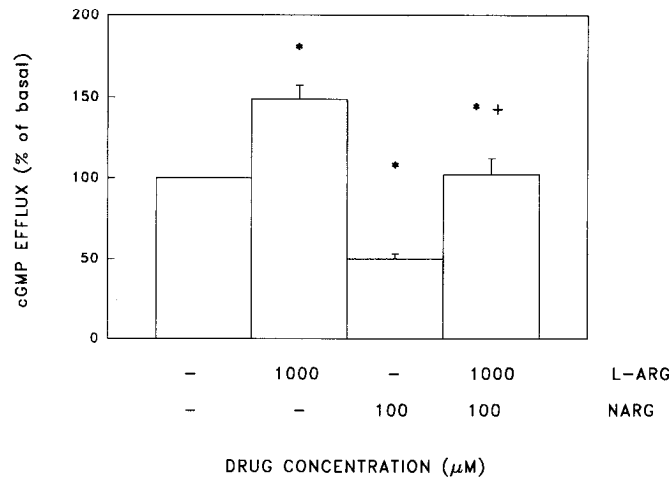


*Figure 7.* Effect of L-arginine on the NARG-induced decrease of the extracellular levels of cGMP in the hippocampus of freely moving rats. IBMX (1 mM) was perfused all throughout the experiment. L-Arginine was administered for 60 min prior to co-infusion (80 min) with NARG (100 μM). Maximum responses are shown. The values are expressed as percentage of basal (±SEM; $n = 4$–6). *, $P < 0.01$ versus controls and basal samples. +, $P < 0.01$ versus 100 μM NARG.

and Higgs, 1991) or by other endogenous agents such as the atrial natriuretic factor (Waldman et al., 1984) or arachidonic acid (Snyder et al., 1984) acting on particulate or soluble guanylyl cyclase, respectively. Moreover, it was recently reported that activation of guanylyl cyclase and cGMP formation could be due to carbon monoxide originated under the catalysis of heme oxygenase-2 (Verma et al., 1993).

The present results demonstrate NO as the main intermediate prior to cGMP generation in the hippocampus of the waking rat. Very recently, Valtschanoff et al. (1993) studied the distribution and the electron microscopic morphology of NO synthase in rat hippocampus. The enzyme was found to be clearly expressed in a small fraction of neurons, most of which appear to be interneurons. The NO synthase–positive neurons were particularly concentrated in the pyramidal layer of the subiculum, in the subgranular zone of the dentate gyrus, and in the pyramidal layer of CA3, although cells in the pyramidal layer of CA1 and CA2 were also stained.

*NO donors.* A number of compounds, including some clinically used nitrovasodilators, are able to generate NO and are now commonly used as donors of NO to tissues and cells. We chose hydroxylamine and SNAP, two drugs reported to raise cGMP levels in rat cerebellar slices (Southam and Garthwaite, 1991). These drugs offer NO by two different mechanisms: catalase and probably other metalloproteins containing heme or flavin moieties present on the target cells are involved in the conversion of hydroxylamine to NO (Waldman and Murad, 1987), while SNAP can spontaneously generate NO by hydrolysis. The hydroxylamine-induced cGMP response displays tissue specificity possibly due to differences in the distribution of the catalyzing enzymes (Waldman and Murad, 1987). The finding that in rat hippocampus hydroxylamine produced a sustained increase in the extracellular levels of cGMP suggests that in this area the drug can easily be transformed into NO. The similar effect produced by SNAP guarantees that the cGMP response caused by hydroxylamine is due to NO produced in the hippocampal tissue.

The results obtained with the two generators of NO clearly show that reverse microdialysis represents an appropriate way to supply NO to selected areas of the brain or to peripheral tissues in order to activate guanylyl cyclase. The increase of cGMP reflects the expected activation of guanylyl cyclase by the NO directly produced from SNAP hydrolysis or metabolically originated from hydroxylamine.

*Effects of NMDA receptor activation on the NO/cGMP pathway.* Activation of NMDA receptors is known to raise cytosolic $Ca^{2+}$, due largely to influx through the receptor-operated ion channel (Mayer and Miller, 1990). In the CNS this $Ca^{2+}$ influx has been shown to stimulate NO synthase (Garthwaite et al., 1988; Knowles et al., 1989). One of the main actions of NO is to activate the soluble form of guanylate cyclase and so increase the levels of cGMP in target cells.

This chain of reactions has been particularly studied in the cerebellum (Bredt and Snyder, 1989; Garthwaite et al., 1989; Wood et al., 1990; Raiteri et al., 1991; Wood, 1991). Actually the *in vitro* or *ex vivo* measurement of cerebellar cGMP levels has been exploited as a functional response linked to NMDA receptor activation.

Preliminary results with adult rat hippocampal slices (East and Garthwaite, 1991) reported a cGMP response linked to NMDA receptor activation, which was however very weak as compared to that in cerebellum. Such a limited cGMP response must have hampered studies of the NO/cGMP system in the adult hippocampus. Indeed, only one recent report shows a consistent increase of the cGMP levels in the hippocampus of adult rats killed by microwave irradiation following intrahippocampal injection of NMDA (Wood et al., 1992). Therefore, the findings that the extracellular basal levels of cGMP together with their drug-induced up- and downmodulations could be easily monitored in the dialysate hippocampus of adult rats have been quite surprising.

Infusions of NMDA caused dose-related elevations of cGMP extracellular levels (Figs. 3, 4). Accordingly, the cGMP response was sensitive to the selective NMDA receptor recognition site antagonist D-AP5. Moreover, the raise of cGMP caused by 500 μM NMDA was totally abolished by 10 μM NARG, clearly showing that, in the hippocampus of the living adult rat, activation of NMDA receptors is linked to NO production and synthesis of cGMP.

The basal outflow of cGMP was not diminished by D-AP5 (Fig. 4) or by the NMDA channel antagonist MK-801 (not shown), suggesting the absence of a tonic NMDA receptor activation. In other words, there can be little activation of NMDA receptors by spontaneously released excitatory amino acids, possibly due to the presence of $Mg^{2+}$ ions blocking the receptor channel in the resting membrane.

*Effects of phosphodiesterase inhibition.* It has long been known that the hippocampus possesses a very efficient mechanism to regulate cGMP levels, since in this area the phosphodiesterase activity is about 10-fold higher than in cerebellum (Greenberg et al., 1978). Accordingly, the continuous perfusion through the dialysis probe of the phosphodiesterase inhibitor IBMX (1 mM) produced a pronounced (eightfold) raise of cGMP extracellular levels. The result suggests that, under basal conditions, the nucleotide is produced in large excess that is almost totally split by phosphodiesterase, although a portion escapes the hydrolysis and can be found in the extracellular fluid. The functional significance of such an efficient removal of cGMP remains to be established. The observation that the IBMX-induced elevation of cGMP remained stable over time does not seem to favor the presence of sensitive negative feedback mechanisms. The idea seems to be strengthened by the ability of the NO synthase substrate L-arginine to increase cGMP formation even during IBMX infusion (Fig. 7). This finding also suggests that in the hippocampus of freely moving rats the endogenous level of L-arginine is not saturating for NO synthase.

It was observed that the levels of cGMP increased before the onset of drug-induced convulsions (Mao et al., 1974; Wood et al., 1982; McCaslin and Morgan, 1986). Moreover, stable cGMP derivatives were found to induce epileptic activity in pyramidal neurons (Hoffer et al., 1977). A role for cGMP in the genesis of epileptogenic activity has recently been proposed (De Sarro et al., 1993). However, during 3 hr of intrahippocampal infusion with 1 mM IBMX, no significative changes in the behavior of the animals were observed, in spite of the large cGMP response. Infusion with the NO donors hydroxylamine or SNAP, which potently increased cGMP formation, could not induce epileptic activity either. Seizures were instead clearly observed during NMDA administration at concentrations producing cGMP responses much lower than that seen with IBMX. Moreover, the seizures were abolished by the NMDA receptor antagonist D-AP5 but not by NARG, which prevented the cGMP response. Our results suggest that epileptogenic activity seems not to be directly related to cGMP levels in hippocampus.

In a recent review the therapeutic potential of inhibitors of cyclic nucleotide phosphodiesterase in some central disorders, including depression, has been presented (Nicholson et al., 1991). The experimental setup employed in the present work may represent a useful model to evaluate *in vivo* novel drugs acting on cGMP phosphodiesterases.

*Conclusions.* The nucleotide cGMP released in the extracellular space of the adult rat hippocampus can be continuously monitored for long time intervals during microdialysis in the freely moving animal. Although the nucleotide is likely to originate from different pathways, NO synthase and its gaseous product appear of primary importance. Accordingly, the cGMP response can be drastically modulated by drugs related to NO synthase activity, leading to the conclusion that the extracellular levels of cGMP reflect the activity of this enzyme and its modulations in the hippocampus of living animals. Monitoring of cGMP during intracerebral microdialysis offers an interesting model for studying *in vivo* and during long time intervals the NMDA receptor complex and related drugs, activators, and inhibitors of NO synthase, NO donors and scavengers, as well as drugs acting at the nucleotide phosphodiesterases.

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