The Influence of Nitric Oxide on Perigeniculate GABAergic Cell Activity in the Anaesthetized Cat

C. Ravdulla1, R. Rodriguez, S. Martinez-Conde, C. Acula and J. Cudeiro1
Lactóricornis de Neuroniina y Comptul Compleraional (Unidad asociada at C.S.I.C. Instituto Cajal), Complejo Hospitalario Unversitario, Universidad de Santiago de Compostela, Spain
1Dept. De Ciencias de la Salud I (E.U. Fisioterapia) and Unidad de Cirugia Experimental (Neurordiagnosticos Visual), Hospital Juan Canales, Corunna, Spain

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Abstract
We have tested the effect of iontophoretic application of the nitric oxide synthase inhibitor 5-nitroarginine on the activity of a population of 53 perigeniculate (PGN) cells, recorded extracellularly in the anaesthetized paralysed cat. In all cells tested with visual stimulation during 5-nitroarginine application (n = 15), the visually evoked responses were markedly reduced, on average by 63 ± 15%, and there was a reduction in spontaneous activity too. This effect was blocked by co-application of the substrate for nitric oxide synthase, L-arginine, but not by the inactive D-isomer, although application of L-arginine alone was without effect. Pressure application of the nitric oxide donor S-nitroso-N-acetylpenicillamine (SNAP) elevated both visual responses and spontaneous discharge, an effect also seen with a second nitric oxide donor, sodium nitroprusside (n = 10). This nitric oxide synthase inhibitor 5-nitroarginine was applied to a sub-population of seven cells and it selectively depresses NMDA mediated excitation (reduction 80 ± 34%) with little or no effect on the excitation mediated by a-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) or quinqueratate (effects not statistically significant), and it had no effect (n = 7) on excitation mediated by the metabotropic agonist (1S,3R)-1-aminoacycloptane-1,3-dicarboxylic acid (ACPD). Furthermore, application of SNAP also increased the magnitude of excitatory responses mediated by kainate receptors. On a different population of seven cells, application of the new NO donor diethylnitro-nitric oxide (DEA-NO) enhanced the actions of NMDA without an effect on responses to AMPA. These effects are qualitatively and quantitatively similar to those we have previously described for X and Y type cells in the dorsal lateral geniculate nucleus (dLGN), despite the known opposite effects of acetylcholine (ACh) application in the dLGN and PGN (ACh is co-localized with nitric oxide synthase at both sites). We propose that within the PGN nitric oxide acts to enhance transmission utilizing NMDA receptors selectively (thereby interacting with the globally inhibiting affect of ACh at this site) to enhance visual responses, reducing or removing the non-specific inhibitory drive from PGN to dLGN seen in the spindling activity of slow-wave sleep. These effects will act in concert with the facilitatory actions of both ACh and nitric oxide within the dLGN proper, and will thereby enhance the faithful transmission of visual information from retina to cortex.

Introduction.
1 The perigeniculate nucleus (PGN), the visual component of the thalamic reticular complex, is a major intrinsic source of GABAergic innervation to the dorsal lateral geniculate nucleus (dLGN). The cells in the PGN have been shown to be insensitive to NMDA, and they send their axons back into the thalamus, innervating both relay cells and local GABAergic interneurons (Fitzpatrick et al., 1984; Montero and Singer, 1984; Kusvit et al., 1987). The PGN receives visual input both from collaterals of dLGN relay cells (Atkin et al., 1978; Ferster and LeVay, 1978; Friedlander et al., 1971; Alkison and Lindstrom, 1981) and from collaterals of the giant descending corticocortical projection to the dLGN (Pujol, 1975, 1977; Boyan and Henry, 1984; Roberts, 1984). This pattern of connectivity allows PGN cells to mediate the recurrent inhibition previously shown to operate on dLGN relay cells (Olley and Rees, 1973; Duhon and Cleland, 1977; Lindstrom, 1982). In addition, PGN cells receive a number of non-visual modulatory inputs, including cholinergic fibres from the prearcuate region of the brainstem, which also innervate the dLGN (Kunos et al., 1981; Alkon and Le, 1982; De Luca and Singer, 1987; Serriade et al., 1987; Bickford et al., 1993; Fuente and Eyel, 1993).

Phenomenological investigations have demonstrated that acetylcholine (ACh) markedly increases the discharge rate of dLGN relay cells while PGN cells, like the intrinsic inhibitory interneurons within the dLGN, are inhibited (Fallon et al., 1976; Goddard, 1978).
Materials and methods

Animal preparation, recording and iontophoretic application of drugs

Experiments were carried out on adult cats anaesthetized with a mixture of N2O (70%), C2H2 (30%) and halothane (0.1-5%) and paralyzed with gallamine triethiodide (10 mg/kg). Experimental procedures for preparation and maintenance of cats and visual simulation have been described previously (Cudmore et al., 1995).

Seven-barrel micropipettes were used for recording single-unit activity and iontophoretic application of drugs in the PGN. Drug barrells contained a selection of the following solutions: N-acetyl-serotonin (5 M for extracellular recordings), 5-amino-A-Arg (10 mM, pH 6.0), 5-amino-v-Arg (15 mM, pH 6.0), N,N,N-tris-(4-aminobenzyl)-L-glutamate (L-NOArg, 10 mM, pH 6.0), sodium nitroprusside (SNP, applied by pressure ejection, 10 mM), 5,7-dihydroxytryptamine (5,7-DHT), 6,7-dihydroxytryptamine (6,7-DHT) and sodium nitrite (SNP, applied by pressure ejection, 30 mM). Dihydroxy-niacinamide complex-sodium (DHA-NO, 10 mM, pH 8), NMDA (5 mM, pH 8), 2-amino-5-phosphonosaliclate (APV, 25 mM, pH 8), 2-amino-5-phosphonovalerate (APV, 25 mM, pH 8), 1,2-amino-3-hydroxy-5-methyl-4-isoxazolopyridin-3-carboxylic acid (AMPA, 15 mM, pH 8), quisqualate (QUS, 25 mM, pH 8), N-methyl-D-aspartate (NMDA). 1,3-D-glutamate (ACPD, 50 mM, pH 8), glutamate, 0.3 M, pH 4.5) or penicillin silver blue (PAS, 0.5 M sodium acetate solution for histological counterstaining).

Pipettes tips were bent back to diameters ranging from 3 to 10 pm, and each drug barrel was subject to a constant retention current of 5-25 nA of appropriate polarity. Intrigroveal cells were identified by sex criteria including anatomic localization, response to visual stimulation, binocularity (Dorn and Christen, 1977; Witzel and Tornstern, 1984). Karlsson et al., 1988). We included in our study cells with anionophoretic application of AcH, because we observed a powerful suppression of cell discharge (see Fig. 1). The experimentally significant attention was immediately and markedly suppressed by application of AcH, the opposite effect to that seen in LGN relay cells. In addition, histological verification of the anatomical location of a number of the recording sites was carried out by injecting horseradish peroxidase (HRP) containing sodium blue for 15-20 min. After removal of the eyes, the brain was sectioned and the blue marks marking the recording sites was determined.

![Graphs and Data](image-url)
NOS inhibitors on excitatory responses evoked by exogenously applied NMDA. We used phenylephrine isometric application of NMDA before and during continuous application of L-NOArg (again alone or in combination with L-Arg or D-Arg). The same protocol was used for the non-NMDA agonist AMPA, QUIS and ACBP. Pressure application of the NO donor SNAP or SNAP aline or in combination with NMDA and SNAP aline or in combination with D-Arg-N04 were also quantitatively examined in the same manner. The magnitude of the drug isometric application current was selected on the basis of initial qualitative observations. In several experiments we included one barrel containing 0.9% NaCl, as a control to eliminate pressure mediated effects; volumes ejected ranged from 5 to 7 μL. It was clear that the incision in the tissue as a result of pressure ejection of both SNAP and SNAP was not isometrically induced by the injection technique, since similar pressure ejection of the saline (vehicle) had no effect on the spontaneous activity of the cells.

Results

The data reported here derive from a total population of 53 cells recorded in PGN. All cells had receptive fields centered less than 15° (degrees of visual angle) from the area ventralis, and stimulating sites were located no more than 1000 μm above the upper border of lamina A of the dLGN.

Effect of application of NOS inhibitors on PGN cells activity

All cells tested showed robust response reductions in visual stimulation during isometric application of the NOS inhibitor L-NOArg (n = 15). This effect was blocked by concurrent ejection of L-Arg and unaltered by D-Arg, the biologically inactive isomer. This is shown in the PSTHs of Figure 2, which illustrate the response of a PGN cell to a drifting sinusoidal grating. In the upper row, the control response to visual stimulation alone is compared with those obtained during continuous isometric ejection of L-NOArg, showing the temporal progression of the inhibitory effect. The visual response was clearly reduced by some 66% after 5 min of application. In the middle row, this cell was tested again after a recovery period, and the effect of simultaneous ejection of both L-NOArg and L-Arg during visual stimulation is illustrated. In this case either the visual response or spontaneous activity (horizontal dashed lines) was altered. Finally, after another recovery period, L-NOArg was applied together with L-Arg. Here again L-NOArg produced a marked reduction of the visual response, reinforcing the view that the suppressive effects of this compound are specific, and are not the result of competitive inhibition of SOM. For this cell, application of L-Arg alone was without significant effect; the visual response being equal to the control level. On average, visual responses were reduced by 65 ± 15% SEM (P < 0.0001, Wilcoxon test). There was no similar degree of suppression of background activity in all cases.

Effect of application of NO donors

The effects of pressure ejection of the chemically untreated NO donors SNAP and SNP on spontaneous activity are shown in Figure 3A. Application of either donor produced a clear increase in discharge rate. This type of effect (P < 0.01, Wilcoxon test) was found in all cell tested (n = 22) and the spontaneous activity was enhanced by ~130%. As can be seen in the PSTHs, the effect of the application outlasted the period of ejection by many seconds. Furthermore, the NO donors (SNAP in this case) were partially able to reduce neuronal activity when they were applied concomitantly with L-NOArg (Fig. 3B). We also tested the application of SNAP on those cells studied with visual stimulation (n = 8). A typical example is illustrated in Figure 4A. The control response is on the left and the PGN cell was stimulated with a sinusoidal drifting grating. On the right, the effect of SNAP application is shown. There is a significant enhancement of the visual response (from 40 ± 2 to 52 ± 3 spikes; P < 0.05 Wilcoxon test). The effect of ejection of L-NOArg on NMDA, AMPA and ACBP resulted responsive in Figure 4B we illustrated the effect of application of the titotic oxide donor SNAP on NMDA evoked responses. Like in effect of visually driven responses (see Figure 4A), SNAP ejection significantly increased responsiveness to NMDA application (2.8 ± 0.2, n = 9, P < 0.05, Wilcoxon test). The effect of ejection of L-NOArg on NMDA, AMPA and ACBP was tested on seven

Fig. 2. The effects of L-NOArg, L-Arg and D-Arg on responses of a NCM cell to visual stimulation. The lower row of PSTHs documents the effect of application of L-NOArg alone on the presentation of a visual stimulus (a 0.6-cd/m² sinusoidal drifting grating; spatial frequency = 0.4 cycles/degree; temporal frequency = 1 c/second). The visual-stimuli presentation was on the left, and the middle PSTH shows activity during repeated presentation of the visual stimulus, 3 min after the beginning the application of L-NOArg. The right PSTH shows a significantly increased inhibitory effect as the L-NOArg-applied is continued for 5 min. The second row of PSTHs again shows a visual stimulus, taken after recovery from the effect of L-NOArg application, and the middle and right PSTHs show no effect from application of L-NOArg, when it is applied in comparison with L-Arg. The third row of PSTHs shows, from left to right, control and responses after 5 and 9 min of continuous application of L-NOArg and L-Arg. Again, the inhibitory effects of L-NOArg are reversed. The lowest row of PSTHs indicates the lack of effect of application of L-Arg alone, even after 5 min of application, the visual responses are entirely unaffected. The dotted horizontal lines represent spontaneous background activity. The bin width was 20 ms and the PSTH duration 1 s, with PSTH shown on the average of 10 trials.

Fig. 3. Effect of L-NOArg, D-Arg and D-Arg on responses of a NCM cell to electrical stimulation. The upper row of PSTHs documents the effect of application of L-NOArg alone on the presentation of a visual stimulus (a 0.6-cd/m² sinusoidal drifting grating; spatial frequency = 0.4 cycles/degree; temporal frequency = 1 c/second). The visual-stimuli presentation was on the left, and the middle PSTH shows activity during repeated presentation of the visual stimulus, 3 min after the beginning the application of L-NOArg. The right PSTH shows a significantly increased inhibitory effect as the L-NOArg-applied is continued for 5 min. The second row of PSTHs again shows a visual stimulus, taken after recovery from the effect of L-NOArg application, and the middle and right PSTHs show no effect from application of L-NOArg, when it is applied in comparison with L-Arg. The third row of PSTHs shows, from left to right, control and responses after 5 and 9 min of continuous application of L-NOArg and L-Arg. Again, the inhibitory effects of L-NOArg are reversed. The lowest row of PSTHs indicates the lack of effect of application of L-Arg alone, even after 5 min of application, the visual responses are entirely unaffected. The dotted horizontal lines represent spontaneous background activity. The bin width was 20 ms and the PSTH duration 1 s, with PSTH shown on the average of 10 trials.
FIG. 3. (A) The effect of application of the NO donors SNAP and SNP on the spontaneous activity of PGN cells. In the upper PSTH a 0.5 pressure ejection of SNAP caused an increase in firing up the cell after ~4 sec, which peaked ~6-10 sec later (not after ~4 sec). (B) The same is typical of the population of 12 cells tested this way. The lower PSTH in (A) shows the effect of application of the NO donor SNP on the spontaneous activity of another PGN cell. A 4 sec pressure ejection of SNP caused a rise in firing, more rapid in onset but of that of SNAP. Each PSTH shows the average of four trials and the bin width was 1 sec. (B) Curve showing the temporal progression of the effect of SNAP blockade on spontaneous activity of another PGN cell (mean ± SEM). After ~1 min of SNAP ejection, the spontaneous discharge declined by ~61%. Application of the NO donor SNAP significantly increased the spontaneous discharge, almost restoring the background activity to control levels.

FIG. 4. (A) The effect of SNAP on the response of a PGN cell as visual stimulation. The left PSTH shows a control response to a drifting sinusoidal grating (spatial frequency = 0.1 cycles/deg; temporal frequency = 1.3 cycles/sec; average of 10 presentations; not width = 1.8 ms). On the right is an example of the visual stimulus that was used, but it was presented during continuous pressurization (2 bar) application of SNAP. (B) Summary bar chart showing the effect of pressure application of the NO donor SNAP on excitatory responses of seven PGN cells to exogenously applied NMDA. (C) Bar chart comparing the maximal excitatory responses of a population of seven PGN cells to NMDA, AMPA, and QUIS (dextro-ben) in the presence of L-NOArg 100 μM, unshaded bars; responses estimated 4 min after L-NOArg onset; 0-9 and C values are the population mean ± SEM, measured over a variable period of time starting and finishing with a significant change in the discharge of the cell. The data were obtained according to the test of Seal et al. (1993) which determines the point of change using the maximum likelihood approach.

In a different group of seven cells, application of the new NO donor DEA-NO enhanced facial discharge, but not in the presence of the NMDA receptor antagonist APV (Fig. 5A). Furthermore, application of DEA-NO enhanced excitatory responses to NMDA (Fig. 6D). Not significantly, this did not affect the response to AMPA (Fig. 6B) in the presence of continuous intrathecal injection of APV to avoid the effects of NMDA receptor mediated spontaneous activity shown in Fig. 6A).
Discussion

These data show that the activity of PGN cells, like that of dLGN cells, can be markedly affected by iontophoretic application of the NOS inhibitor L-NOArg. That such an effect seems to be specifically due to inhibition of NOS synthesis is supported by the following observations. (i) The inhibitory effect of L-NOArg was antagonized by co-application of L-Arg, the biological substrate for the enzyme NOS. (ii) In a similar paradigm, D-Arg, the biologically inactive isomer of arginine, was unable to restore normal activity. (iii) Application of the chemically unrelated NO donors SNP, SNAP or DEA-NO significantly increased both spontaneous activity and visually driven responses. It is known that the sole substrates of NOS in the dLGN/PGN complex is the glutamatergic pathway from the parabrachial nucleus (Backlund et al., 1993; where NADPH-diaphorase, a marker for NOS has been shown to co-localize with ACh; however, the effects of ACh at the two sites are opposite, with enhancement of dLGN activity and suppression of PGN activity (Hixson et al., 1976; Godrindia, 1978; Stills et al., 1983; Eyssel et al., 1986; McCormick & Prince, 1987; Franceschetti et al., 1988). Surprisingly, then, the data presented here show that blockade of NOS synthesis in the PGN produces a potent and relatively selective reduction of NMDA-evoked excitation, just as we have previously demonstrated for cells in the dLGN (Castedo et al., 1996a, 1996b). A direct action of L-NOArg on NMDA receptors is unlikely, as L-NOArg has previously been shown neither to produce any effects on NMDA membrane currents nor affect [³H]MK-801 binding (Jawson et al., 1991). An interesting point arising from our results is the finding that L-Arg (red), while antagonizing the block of NO production, itself produces no further increase in firing. Again this mirrors our observations in the dLGN (Castedo et al., 1994a, b). In a previous study we concluded the possibility that the action of NOS blockade was-NMDA selective only by virtue of the voltage selective nature of NMDA responses, by demonstrating that the effect of NOS blockade was still present when cells were hyperpolarized by exogenous application of GABA (Castedo et al., 1996). Although we have not repeated this experiment here, it seems unlikely that these should exist two separate mechanisms for the enhancement of NMDA responses at the level of the LGN/PGN complex. Furthermore, the inability of DEA-NO in excitatory AMPA responses in the presence of APV suggests an NMDA specific action.

We propose that in normal function (or at least in the experimental conditions outlined here) available NOS; the enzyme producing NO, is fully active, being a rate limiting step in the control of NMDA mediated excitation. The similarity between these results and those obtained in the dLGN proper is at first surprising, given the opposite polarity of the effect of ACh application. Although this suggests a common mechanism of NO action operating throughout the dLGN/PGN complex in the cat, the difference in the action of ACh (red by extension the actions of the parabrachial input) at these two sites must also be accounted for by any explanation for these effects. While the sympathetic pharmacology of the PGN is as yet not fully characterized, it is clear that these cells respond in an extraordinary fashion to the application of the excitatory amino-acids, NMDA, AMPA, QUIS and...
NOS, known to be Ca\(^{2+}\) dependent, may follow the level of activity in the cholinergic fibers of the parabrachial input, as terminal Ca\(^{2+}\) levels fluctuate (Codere et al., 1996), utilizing the increased tonic pool of arginine known to follow from sensory stimulation (Ro et al., 1994). With this in mind it is tempting to speculate further that an increase in NO levels brought about by increased parabrachial activity, during arousing or attentive periods, enhances the NMDA mediated transmission to the PGN via two different pathways: the activity of the relay cell collaterals and input from the visual cortex. Activity in the deep layers of the cortex, from which this input to the dLGNePGN arises, is known to be diminished during sleep and increased during arousal (Lingvist and Hobel, 1981). Transition from slow wave sleep to the awake state is known to be associated with an increase in neurotransmitter release from many brainstem and hypothalamic sites, including enhanced release of ACh, noradrenaline, 5-hydroxytryptamine (5HT) and histamine (for a review see Steriade and McCarley, 1990; McCormick, 1982). Thus a possible action of the parabrachial input would be to enhance cortical input from cells known to have vesicular release fields which are highly oriented and direction specific (Grieve and Silipo, 1995) selectively using NMDA receptors, at the expense of the relay cell feed-forward inhibition (LGN to PGN to LGN) driven by non-NMDA receptor activation, even though relay cell activity can be directly enhanced by both ACh and NO. (The importance of the relay cell collateral pathway is likely to be increased during spindle activity, in the relative absence of ACh and NO.) It is noteworthy interesting to note a recent study of the visual properties of PGN cells which showed that spontaneous activity was suppressed, while visual responsiveness was enhanced, during elevated stimulation of the parabrachium.
(Murphy et al., 1994). In this scenario, the paracrinoid production of which would globally suppress PGN cell activity by means of muscarinic receptors, (perhaps enhancing visual activity with a transient nicotinic activation; see Lee and McCormick, 1995), and with release of NO would allow NMDA receptor activation to be enhanced relative to non-NMDA. We have illustrated these hypotheses in Figure 7.

In summary, the simplest explanation of the data we have presented here is that, as we have previously suggested for cells in the dLG, the actions of NO serve to enhance visual responses of PGN cells, in particular those involving the activation of NMDA receptors.

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Abbreviations

AC, acetylcholine
ACPD, (15,3R)-1-amino-1-cyclopentyl-1,3-dicarbonyl acetic acid
AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
APV, 2-amino-5-phosphatepicolinate
Aγ-R, α-agonists
Aγ-L, α-antagonists
DEA-NO, diethylamine-nitric oxide
dLG, dorsal lateral geniculate nucleus
GAABA, γ-aminobutyric acid
Mas, muscarinic agonists
NOsyn, nitric oxide synthase
PGN, perigeniculate nucleus
PSTH, peri-stimulus time histogram
QUS, quinoxaline
SNAP, S-nitroso-N-acetyl penicillamine
SNP, sodium nitroprusside

References


