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Modulatory Influence of Putative Inhibitors of Nitric Oxide Synthesis on Visual Processing in the Cat Lateral Geniculate Nucleus

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SUMMARY AND CONCLUSIONS

1. Using an in vivo preparation we have examined the actions of two inhibitors of nitric oxide synthase (NOS), N^G-nitro-L-arginine (L-NOArg) and N^G-methyl-L-arginine (L-MeArg), in the feline dorsal lateral geniculate nucleus (dLGN). We compared the responses obtained to iontophoretic application of these substances during visual stimulation with those elicited by visual stimulation alone. The effects of concurrent ejection of L-arginine (L-Arg), the normal physiological substrate of NOS, and D-arginine, the inactive isomer, were tested on these responses.

2. Extracellular application of L-NOArg and L-MeArg produced clear and repeatable effects, consisting of substantial reduction in discharge rate without affecting response selectivity, on 94% of tested cells. These effects were prevented by simultaneous application of L-Arg, which when ejected alone produced no change on visual evoked responses.

3. The data suggest that nitric oxide (NO) is necessary for the transmission of the visual input under normal visual stimulation and show a direct involvement of NO in visual information processing at the level of dLGN, suggesting that its contribution to brain mechanisms is more profound that previously thought.

INTRODUCTION

Nitric oxide (NO), first identified as endothelium-derived relaxing factor, is also an important neuronal messenger molecule (Bredt and Snyder 1992; Garthwaite 1991; Moncada et al. 1991). In vitro experiments have shown a physiological role for NO in the CNS by demonstrating that certain arginine derivatives block glutamate-stimulated formation of guanosine 3′,5′-cyclic monophosphate in the cerebellum (Bredt and Snyder 1989; Garthwaite et al. 1989). NO has been linked to long-term potentiation (LTP) (Izumi et al. 1992; Schuman and Madison 1991) and it has been suggested that NO is produced in postsynaptic structures in response to stimulation of excitatory amino acid receptors, from which it diffuses to act on neighboring cellular elements, probably presynaptic nerve endings and astrocyte processes (Garthwaite 1991). Although there is an increasing interest in the role of NO as an intercellular messenger, very few papers have been published on the role of NO in sensory systems, and usually in vitro techniques have been used. Therefore, in the present paper, we have examined the effect of iontophoretic application of two inhibitors of NO synthase (NOS), the enzyme that produces NO, on X and Y cells in the dorsal lateral geniculate nucleus (dLGN) of anesthetized and paralyzed cats.

METHODS

Experimental procedures for the preparation and maintenance of cats and visual stimulation have been described previously (Sillito et al. 1993). Using multibarrelled pipettes for extracellular recording and iontophoretic drug applications, recordings were made from single neurons in the A laminae of dLGN of 10 cats anesthetized with a gas mixture of 70% N_2O-30% O_2 and halothane (0.1–0.5%) and paralyzed with gallamine (10 $mg \cdot kg^{-1} \cdot h^{-1}$). Each drug barrel of the electrode contained a selection of the following: 3 M NaCl for extracellular recording, L-arginine (L-Arg; 10 mM, pH 6.0), D-arginine (D-Arg; 10 mM, pH 6.0), N^G-methyl-L-arginine (L-MeArg; 10 mM, pH 6.0), N^G-nitro-L-arginine (L-NOArg; 10 mM, pH 6.0), and Pontamine Sky Blue (2% wt/vol in 0.5 M sodium acetate) for histological reconstruction. The typical experimental paradigm was as follows. When a spike was isolated and its receptive field center determined, we checked the linearity of spatial summation using phase-reversing sinusoidal gratings. The results from this, together with information regarding center size, strength of surround antagonism, and the presence or absence of a shift effect were used to categorize the cell as X or Y type (Cleland et al. 1971; Derrington and Fuchs 1979; Enroth-Cugell and Robson 1967). Cells were classified as lagged or nonlagged according the criteria of Humphrey and Weller (1988) and Mastronarde (1987), with particular emphasis given to the latency to half-rise time. All cells had receptive fields within 12° of the area centralis. Responses of dLGN neurons to visual stimulation were assessed using flashing spots of several sizes, moving bars of different lengths, and drifting sinusoidal gratings of several spatial frequencies repeated over a number of trials and varied in a randomized interleaved sequence. The contrast ($L_{max} - L_{min}/L_{max} + L_{min}$) routinely used was in the range of 0.35–0.7, with a mean luminance of 14 cd/m^2. After that we obtained control responses to appropriate visual stimulation, and then we tested the effect on these responses of application of the drugs separately or in combination. The response to drug application was routinely seen within 2–3 min after application was commenced and lasted 7–15 min after cessation of application.

RESULTS

The vast majority of the cells, 30 of 32 (94%), showed marked response reductions to visual stimulation during iontophoretic application of either L-MeArg or L-NOArg, as illustrated in Fig. 1, which shows the responses of an ON center Y cell. Figure 1*A* shows the tuning curve for flashed spots of varied diameter in the control situation (solid line) and the responses during application of L-NOArg (broken line), which resulted in clear reduction of the visual responses to all diameters of stimulus with a concomitant reduction in spontaneous activity as judged from the

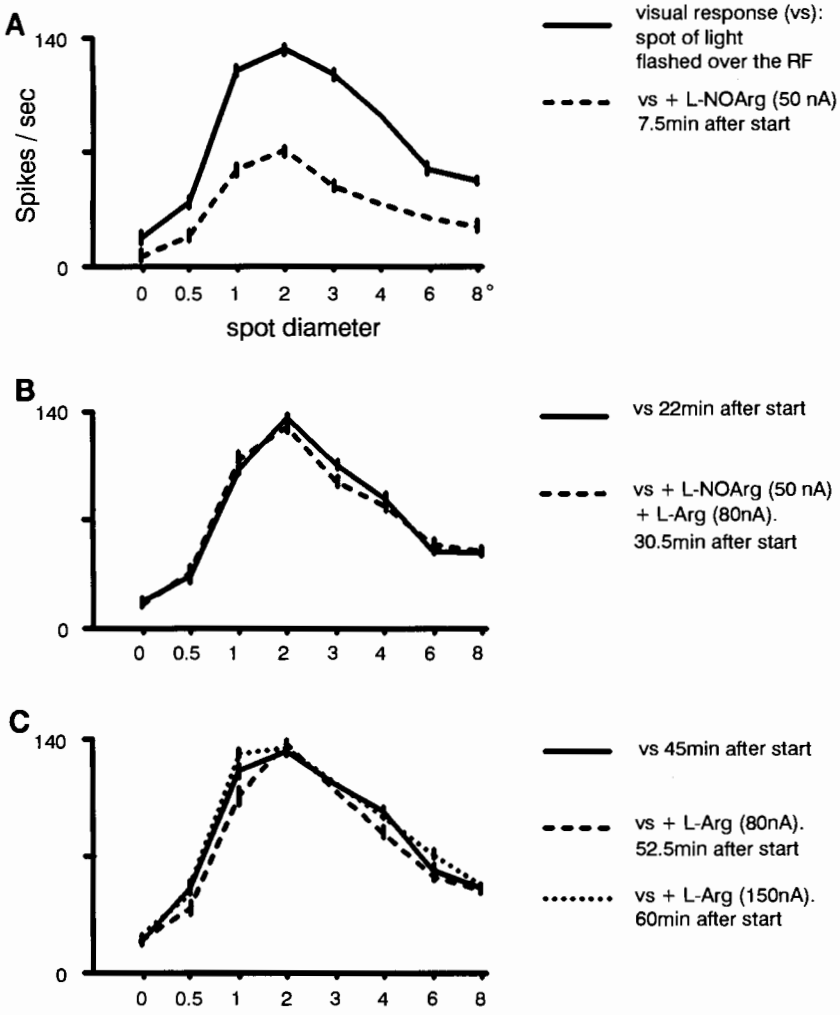

FIG. 1. Tuning curves illustrating the responses recorded from an ON center Y dorsal lateral geniculate nucleus (dLGN) cell using a 7-barreled iontophoretic electrode. Vertical axis of each curve: number of action potentials (spikes per second). *A*: control responses (solid line) when the cell was stimulated by flashing spots of light placed over the receptive field center. Eight different diameters were presented in a randomized interleaved sequence. Broken line: cell discharge with visual stimulation as before, but with simultaneous, continued application of N^G-nitro-L-arginine (L-NOArg). The reduction of the visual responses is very evident. Small vertical bars: mean ± SE. The ejection current is indicated in brackets. All times relate to time in minutes after the start of the experimental run. *B*: same sequence, but 10 min after termination of the L-NOArg ejection (solid line). After this recovery, L-NOArg and L-arginine (L-Arg) were ejected concomitantly (broken line), the former completely blocking the inhibitory effect of L-NOArg. *C*: solid line: recovery after drug application. Broken line: responses during ejection of L-Arg. This drug, applied alone, had no effect on visual evoked responses (broken line), even when a high ejection current was used (· · ·). During each test, recovery, or drug application, all visual stimuli were repeated 8 times (i.e., 8 trials), taking ~4.5 minutes per test.

"zero" stimulus condition (randomly interleaved within in each visual test sequence). The "optimum" spot diameter for the cell was unchanged, indicating no specific alteration in the center versus surround antagonistic interaction. If these observations genuinely follow from a physiological effect of NO, then application of L-Arg, the physiological substrate of NOS, should restore normal activity. This is the case illustrated in Fig. 1*B*. After a return to predrug control response levels (solid line), ejection of the two drugs together (L-Arg + L-NOArg, broken line) using the same level of application current as was used in Fig. 1*A* resulted in a complete loss of the L-NOArg-induced inhibition, including effects on spontaneous activity. The specificity of this blockade is noteworthy in that application of L-Arg completely abolished the inhibitory effects of whichever inhibitor of NOS was applied but was itself without excitatory effects when applied alone, even when applied with ejection currents markedly exceeding those required to block the activity of the NOS inhibitors (Fig. 1*C*). Such a response profile suggests that in the experimental situation outlined here, the normal operating system by which NOS produces NO from L-Arg is fully active. Other specific receptive field properties, such as selectivity for the length of a moving bar and spatial frequency preference, were also unaltered during the drug application. A second example is shown in Fig. 2, which illustrates the responses of an OFF X cell to a similar experimental paradigm. The peristimulus time histograms of the responses to drifting sinusoidal gratings of varied spatial frequency are shown: in this case application of L-MeArg resulted in a decrease in firing in both background and visually evoked firing (Fig. 2*B*), which was blocked by concomitant application of L-Arg (Fig. 2*C*), which again was itself without effect (Fig. 2*A*). Once more the selectivity of the visual responses of the cell was unchanged. In a number of cells ($n = 5$) we applied D-Arg, the biologically inactive isomer of L-Arg, using application currents equal to those used to apply L-Arg. In all cases the effects of the NOS inhibitors were unaffected. We found no differences in the responses to NOS inhibitors between dLGN cell types.

DISCUSSION

These data are in keeping with the growing evidence that NO serves as a regulatory molecule in cellular communication in a number of different types of tissue (Bredt and Snyder 1992; Garthwaite 1991; Vincent and Hope 1992). In the CNS the best-understood function of NO appears to be that of a retrograde intercellular messenger, which is formed in response to activation of excitatory amino receptors (Garthwaite et al. 1988), and it has been linked to LTP (Izumi et al. 1992; Schuman and Madison 1991). Our re-

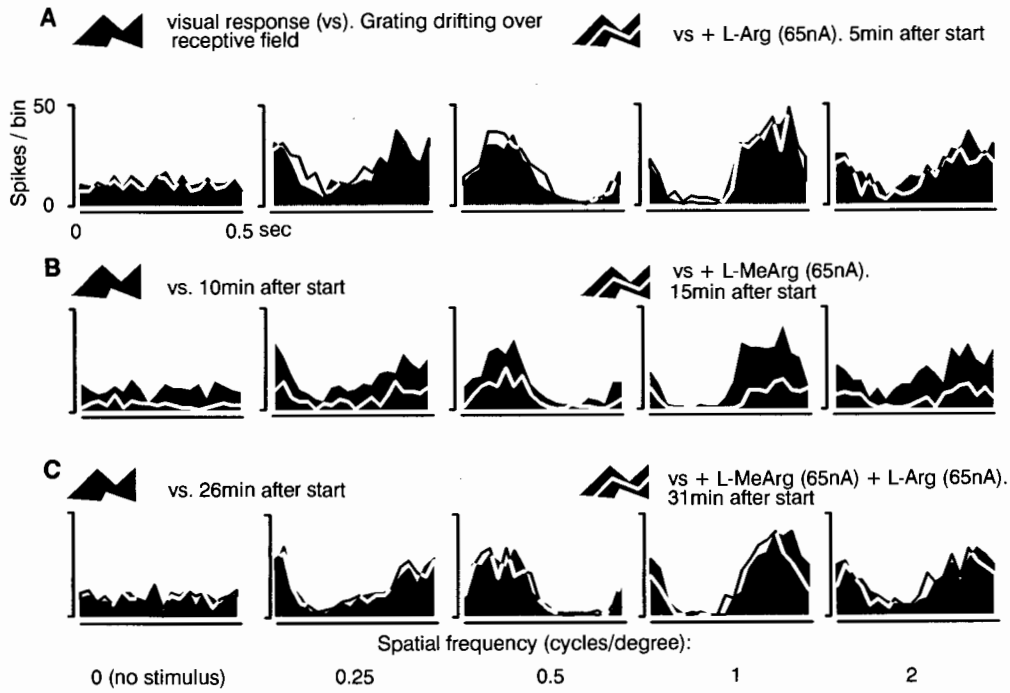

FIG. 2. Peristimulus time histograms illustrating the responses of an OFF X cell to drifting sinusoidal gratings of varied spatial frequency. For each presentation 5 cycles of the stimulus were drifted over the receptive field. *A*: control responses of the cell without stimulation and to gratings of 0.25, 0.5, 1, and 2 cycles per degree (solid histograms), and repeated in the presence of iontophoretically applied L-Arg (line histograms). *B*: repeated control responses after ejection of L-Arg (solid histograms), and the effect of application of the nitric oxide synthase (NOS) inhibitor N^G-methyl-L-arginine (L-MeArg) (line histograms). *C*: solid histograms: repeated control responses. Line histograms: responses during combined application of both drugs. The visual stimulus was a sinusoidal grating drifted across the receptive field at a temporal frequency of 2 Hz and was 10° in diameter. Again, all times relate to time in minutes after the start of the experimental run. Bin size = 25 ms.

sults support a new role for NO—that of enabling the transfer of visual information at the level of the cat dLGN, because application of both L-NOArg and L-MeArg, inhibitors of NOS, reduced responses elicited by visual stimulation, and the effects of the blockers were selectively antagonized by simultaneous application of L-Arg. Although there exists the possibility of a nonspecific action of L-NOArg and L-MeArg (Archer and Hampl 1992; Rosenblum et al. 1992), the antagonism of the inhibitory effect of these compounds by L-Arg and the lack of effect of D-Arg argue for a highly selective blockade of NOS. This NO synthesizing system seems to be fully active, because the ejection of L-Arg was itself without any effect even when it was applied with ejection currents greatly exceeding those required to block the activity of the NOS inhibitors. Although the question of a presynaptic versus postsynaptic locus of activity must remain open, there is recent evidence that the release of NO in vitro onto thalamocortical neurons dampens oscillatory neuronal activity, indicating a rapidly diffusing signaling mechanism acting postsynaptically (Pape and Mager 1992). Although the cells within the dLGN do not appear to contain significant levels of NOS, immunocytochemical evidence has demonstrated a high level of NADPH diaphorase staining [a highly selective marker for NOS, (Dawson et al. 1991)] in fibers innervating the thalamus (Vincent et al. 1983), where it appears to be colocalized in cholinergic fibers arising from the mesopontine tegmental nuclei in the brain stem (Bredt and Snyder 1992; Snyder and Bredt 1991; Vincent and Hope 1992), which form synapses with both relay cells and interneurons (De Lima et al. 1985). Indeed, the most recent findings have demonstrated that the cholinergic axons from the brain stem are the exclusive source of NO in the cat dLGN (Bickford et al. 1993). This evidence, taken together with our data, suggest a new model for NO action in the CNS, acting postsynaptically. It is tempting to speculate that this pattern of connectivity allows the brain stem, utilizing a combination of acetylcholine and NO, to exert a powerful facilitatory influence over the transfer of visual information to the cerebral cortex, globally enhancing the dLGN relay cells' activity but without affecting response selectivity.

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