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An unusual effect of application of the amino acid L-arginine on cat visual cortical cells

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IONTOPHORETIC application of L-arginine (L-Arg) resulted in a profound decrease in visually elicited and spontaneous activity in 22 of 77 (29%) cells in area 17 of the anaesthetized/paralysed cat. Duration was long, and cells did not recover pre-application activity levels, indicating permanent decline. This effect was obtained without change in the extracellularly recorded waveform, demonstrating that this did not result from depolarization block. In the remaining 55 cells, application of L-Arg alone, at levels capable of eliciting inhibition as described above, was without effect. In 29 cells, L-Arg application was able to reverse the effect of inhibition of nitric oxide (NO) production. Populations of cells showing the depressive effect described above and those affected by NO modulation levels were mutually exclusive.

Key words: L-Arginine; Cat visual cortex; (Neuro)modulation; Nitric oxide; Nitric oxide synthase

Introduction

In the search for physiological roles for the gaseous neurotransmitter nitric oxide (NO) in the CNS, many different techniques and neuronal systems have been examined. NO is formed by the action of the enzyme nitric oxide synthase (NOS) on its biological substrate, the amino acid L-arginine (L-Arg). Within the CNS, NOS exists in a number of different isoforms: that most intimately involved in neuronal function is thought to be neuronal NOS (nNOS).[1–4] We studied the visual cortex of the anaesthetized cat in an attempt to determine the effects of compounds thought to operate on the NO system and on NOS. While many cells responded to the application of both an antagonist of nNOS and L-Arg in a manner consistent with other recent observations,[5–8] an unusual effect of L-Arg was observed in a large proportion of cells.[9] Here we report this unusual, depressive effect, which was observed on approximately 30% of cells tested, and we discuss these findings in the context of the wider aspects of NO and NOS in CNS tissue.

Materials and Methods

Nineteen cats were anaesthetized with halothane (0.1–5%) in NO_2 (30%) and O_2 (70%), and paralysed with gallamine triethiodide (10 mg kg^{-1} h^{-1}). EEG, ECG, expired CO_2 and temperature were monitored and maintained continuously, adjusting anaesthetic levels to maintain a state of light anaesthesia. Further details are given in Ref. 7. Single units from primary visual cortex were recorded extracellularly using multibarrelled glass micropipettes filled with a combination of the following drugs: NaCl (3 M for recording), L-Arg (10 mM, pH 6.0), L-lysine (10 mM, pH 6.0), L-NOArg (10 mM, pH 6.0), diethylamine-nitric oxide (DEA-NO, 10 mM, pH 8.0), or Pontamine Sky Blue (PSB, 2% w/v, in 0.5 M sodium acetate solution, for histological reconstruction). When not in use drug barrels were subjected to a retention current of 5 to 25 nA of appropriate polarity. L-Arg was purchased from Sigma chemicals and two different batches were used, both capable of producing the depressive effect described below. Drug application currents were selected on the basis of initial qualitative studies, and were generally held in a range which did not saturate responses. Single unit data were collected and visual stimuli produced under computer control (Visual Stimulation System, Cambridge Electronic Design, Cambridge, UK).[7] Stimuli were viewed

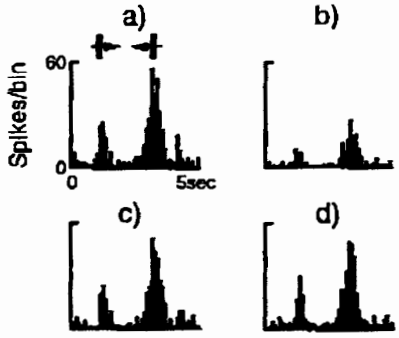

FIG. 1. peristimulus time histograms (PSTH) documenting the effect of manipulation of nitric oxide (NO) levels. (a) Control visual responses to a bar stimulus drifted back and forward (diagram above the records) across the receptive field at the preferred orientation. (b) Reduced responses seen during continuous iontophoretic application of L-NOArg, 70 nA. (c) Co-application of L-NOArg with L-Arg, blocking the L-NOArg effect. (d) Lack of effect of L-Arg applied iontophoretically alone. PSTH are in 100 ms bins.

monocularly through the dominant eye for each cell under test. Our basic paradigm was to establish control responses to a drifting bar stimulus, moving backward and forward through the receptive field, at the optimal orientation. Subsequently, this procedure was repeated in the presence of the compound or compounds of interest. We also examined drug effects on spontaneous activity. Typically, responses were averaged over 10-20 stimulus presentations and assessed from the accumulated count in peristimulus time histograms (PSTH).

Results

In our sample of 77 cells two observations were of note – in some 25 cells application of the NOS inhibitor L-NOArg (which is a competitive inhibitor for the L-Arg site) reduced visual responses, an effect which was reversed by co-application of L-Arg (Fig. 1). In each case, however, application of L-Arg alone was without effect (Fig. 1d). This and other aspects of the pharmacology of NO-mediated responses are dealt with in a separate study (manuscript submitted for publication).

Figure 2 shows control PSTH of the visual responses of a cortical cell (Fig. 2a) prior to drug application, contrasted with the reduced responses seen during application of L-Arg (Fig. 2b). Both visually elicited and spontaneous activity are greatly depressed. Insets to the figure show the recorded analogue waveforms of the action potential, taken both before and during L-Arg application. Clearly there is no shift in the shape of the waveform which would be consistent with a loss of activity resulting from, for example, a depolarization blockade. The histogram in figure 2c summarizes the magnitude of

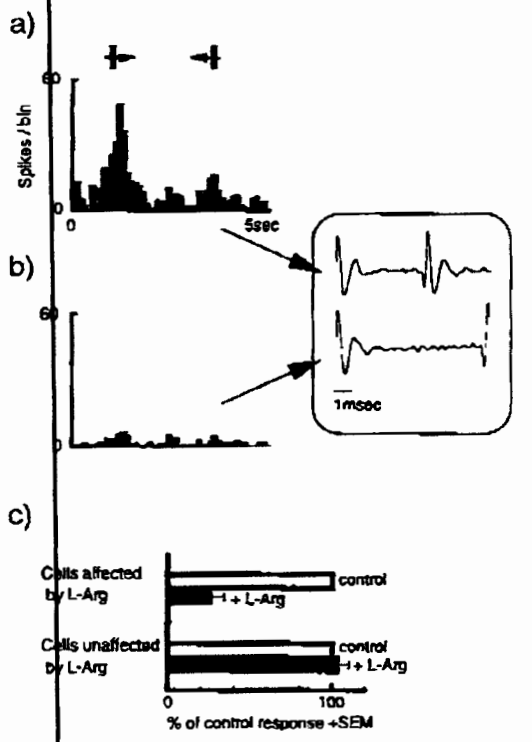

FIG. 2. peristimulus time histograms (PSTH) documenting the suppressive effect of L-Arg. (a) Control visual responses, as in figure 1. (b) Responses during continuous application of L-Arg. Bin width 100 ms. To the right are representative waveforms of the spike, taken from the filtered analogue data, showing no change in shape during L-Arg induced suppression. (c) Summary histogram showing responses of cells affected by L-Arg (upper panel) prior to and during L-Arg administration. The lower panel shows a similar histogram for the cells unaffected by L-Arg administration. All responses are normalized to the pre-drug administration control level.

L-Arg-mediated inhibition (on average, after 3 min of L-Arg application, responses were reduced by 75 ± 6% (s.e.m.), and compares this with the lack of effect seen in the remainder of the population. Further examples are given in figure 3. This shows control PSTH of the visual response of two cortical cell (columns: upper simple cell, lower complex cell) prior to drug application followed by the responses during application of the NOS inhibitor L-NOArg. In each case visual responses are unaffected. Column (c) shows that application of the amino acid L-lysine (with L-Arg, one of the three basic amino acids at physiological pH),[10] was also without effect. In contrast the responses seen during application of L-Arg (column d) were greatly reduced.

It is of note that cells affected by L-NOArg were never affected by L-Arg in the manner described above (Figs 2,3) and *vice versa* (although the two cells types were encountered in the same animals and in the same penetrations). Control responses (Fig. 4a)

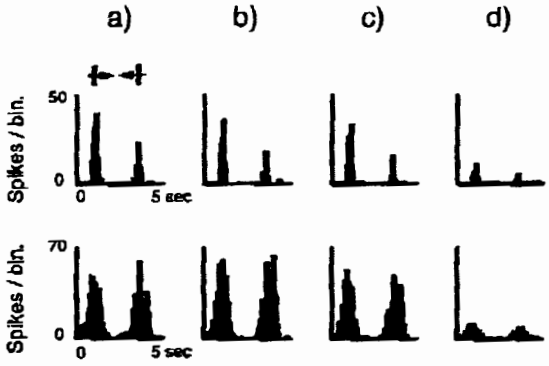

FIG. 3. Responses of two cortical cells – upper row, a simple cell in layer IV, lower, a complex cell in layer V. Column (a), control responses, (b) the lack of effect of iontophoretic application of L-nitric oxide arginine. (c) Lack of effect of application of the related amino acid L-lysine. (d) Suppressive effect of L-Arg. Bin size 100 ms.

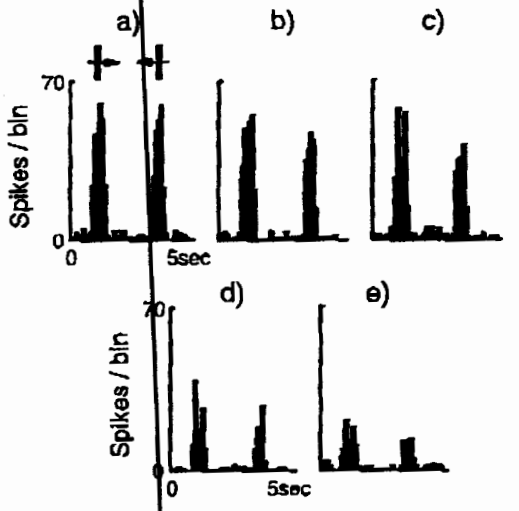

FIG. 4. Ineffectiveness of NO manipulation cells suppressed by L-Arg. (a) Control visual responses. (b) Lack of effect of application of the NO donor DEA-NO. (c) Lack of effect of application of L-NOArg. (d,e) Suppressive effect of application of L-Arg with 20 nA current (d) and 60 nA (e). Bin size 100 ms.

were unaffected by application of the NO donor DEA-NO (Fig. 4b) or L-NOArg (Fig. 4c) but were markedly reduced by L-Arg (Fig. 4d) with a 20 nA application current, and where 60 nA reduced visual responses to near zero (Fig. 4e).

In all 22 cells (including those illustrated in Figs 2 and 4) the profound depressive effect greatly outlasted the period of application, such that even after a period of many minutes (up to 1 h) activity remained diminished. The effect was relatively slow in onset, taking 2–3 min to reach a peak. Cells of all visual types were affected, and histological reconstruction showed that they were found in all laminae, II–VI.

Discussion

The basic finding of this study is that a highly significant proportion of cat visual cortical cells (some 29%) respond to the application of L-Arg with a profound and prolonged depression of spiking activity. We can offer several possible mechanisms by which such an effect may be derived. First, in the neuropil around cells inhibited by L-Arg, locally available NOS may be inactive at the time of application of L-Arg, because of local variation in the availability of native L-Arg. Thus when L-Arg is applied there is a rapid production of NO, which inhibits the cell. We reject this hypothesis on two grounds, first, application of DEA-NO did not inhibit this type of cell and therefore inhibition by production of NO from exogenously applied L-Arg is unlikely. Second, only cells whose activities are depressed by L-Arg are in this group, (as above, suggesting an inhibitory effect of NO generation) whereas in the group of cells affected by NOS blockade using L-NOArg by far the most prominent effect of NO was seen to be excitation. A second possible mechanism relies on the fact that, in the mammal, NOS is produced in several isoforms, of which nNOS is thought to be the most abundant in brain.[1–4] L-NOArg is several times more potent an inhibitor of nNOS than other compounds.[11] L-NOArg is a more potent blocker of nNOS than of inducible NOS.[11] Were this type of NOS to be activated here, which is not readily blockable by L-NOArg, application of excess L-Arg could result in overproduction of NO, which under some conditions is cytotoxic.[1–4] Since cytotoxicity results from a hyperactivity of spiking, the absence of an excitatory effect of DEA-NO again argues against this, as does the absence of any L-Arg mediated excitatory responses. Since the relative effectiveness and zone of spread of the two compounds are not known (although theoretically NO released from a point source is thought to act on a sphere of neuropil of 200 μm in diameter)[12] it remains possible that diffusion of L-Arg over a large area results in a remote release of NO, acting on remote cells or processes. With this in mind it is interesting to note that a recent study showed that NO could enhance the inhibitory postsynaptic currents induced by activation of α-aminobutyric acid ($GABA)_B$ receptors.[13] Data from hippocampal pyramidal cells have been shown that such $GABA_B$ receptors are located distally, on more remote dendritic processes,[14] and application of agonists for such receptors have been shown to depress visually elicited activity in cat visual cortical cells.[15] Thus a remotely induced $GABA_B$-mediated inhibition can account for the data we present here,

but we must still hypothesize a clear division of cell types, along some unknown axis. Finally, L-Arg, a basic amino acid involved in numerous biochemical processes *in vivo*, possibly given in amounts greatly exceeding normal physiological levels, may result in some form of metabolic toxicity. Application of L-lysine (in common with L-histidine and L-Arg, being one of the amino acids with charged NH_3 groups at physiological pH, i.e. basic amino acids)[10] with similar iontophoretic current levels, did not alter spiking activity in any cell. Against the idea of such metabolic toxicity however, is the fact that this effect was seen in only 29% of cells, and never in a cell whose activity was affected by NO-modulating compounds.

We must therefore conclude that there is some as yet unknown difference between three cortical cell groups – cells affected by NO modulation, cells suppressed by L-Arg, and cells unaffected by either. With this in mind it is interesting to point out that evidence from primate visual cortex suggests that NOS is localized in cortical regions which are also rich in cytochrome oxidase,[16] and evidence has been provided recently to suggest a parcelation of cat visual cortex with a blob system of cytochrome oxidase, similar to that in the primate.[17] Our relatively small sample size prohibits a clear visualization of parcellation – cells depressed by L-Arg were of all cell types, and found in all laminae, as were cells affected by NO modulation. We are currently investigating the organization of these systems, using long tangential penetrations through the cortex to examine any possible columnar organization, and the synaptic/biochemical mechanism of this profound L-Arg-mediated effect.

Conclusions

Application of the L-Arg to some 29% of cells in the primary visual cortex of the cat can cause a pronounced and prolonged depression of spiking activity. This effect appears to be unrelated to the NO system, since compounds known to modulate NO levels, with the exception of the L-Arg, have no effect on these cells, and cells in which NO modulation alters spiking never show this L-Arg mediated depression. Cells which demonstrated this novel effect were found in all laminae, and were of all types. As yet this effect remains unexplained.

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General Summary

L-Arginine is the biological substrate of the enzyme nitric oxide synthase (NOS), which produces the gaseous neurotransmitter nitric oxide (NO). We have demonstrated an unusual effect of application of L-Arg in a subpopulation of cortical cells, resulting in prolonged depression. Such cells are unresponsive to other compounds thought to alter NO levels, and we suggest that this response is unlikely to be mediated by manipulation of the NO system, although this remains a possibility. Alternatively, this may be a toxic effect of exposure to L-Arg in possibly pharmacological rather than physiological amounts. A second population of cortical cells was affected by NO manipulation in a more predictable manner. The population of cells affected by NO manipulation and those inhibited by L-Arg are mutually exclusive.