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COMMUNICATIONS

Effects of L-nuciferine on kainate, *N*-methyl-D-aspartate and acetylcholine excitation of cat spinal neurons

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Duggan et al (1973) first examined the possibility that the aporphine alkaloid, 1-5,6-dimethoxyaporphine (L-nuciferine), might be a specific excitatory amino acid antagonist. However, in studies on Renshaw cells in the rat spinal cord they found that L-nuciferine did not differentiate between the excitatory effects of L-glutamate and acetylcholine. In contrast, more recent data from other laboratories indicate that L-nuciferine depresses amino acid-induced excitation but not acetylcholine-induced excitation on neurons in the feline ventro-basal thalamus (Ben-Ari & Kelly 1975) and pigeon optic tectum (Felix & Frangi 1977). Moreover, results from two laboratories suggest that the alkaloid may preferentially act on L-glutamate receptors (Felix & Frangi 1977; Polc & Haefely 1977).

In view of these conflicting reports, it seemed important to re-examine the effects of L-nuciferine on the action of excitants on spinal neurons. Hence the effects of the alkaloid were determined on the excitatory responses induced by acetylcholine and amino acids on Renshaw cells and dorsal horn neurons in the cat. The amino acids used were kainate and *N*-methyl-D-aspartate (NMDA) because it has been suggested that kainate and NMDA act on 'glutamate-preferring' and 'aspartate-preferring' receptors respectively (Johnston et al 1974; McCulloch et al 1974).

Experiments were performed on cats anaesthetized with pentobarbitone sodium (35 mg kg^{-1}, i.p. initially, supplemented by 5 mg kg^{-1}, i.v. when required). The spinal cord was exposed by lumbar laminectomy, transected at L1 and covered with paraffin oil (37 °C). The cut central ends of L7 and S1 ventral and dorsal roots were stimulated to aid identification of Renshaw cells and dorsal horn neurons respectively. Extracellular recordings were made via the centre barrel (4 M NaCl) of a multibarrel microelectrode. The outer barrels contained aqueous solutions of the substances to be administered electrophoretically, These were: *N*-methyl-D-aspartate Na (NMDA, 0·05 M in 0·1 M NaCl, pH 7), kainate Na (0·02 M in 0·13 M NaCl pH 7) acetylcholine chloride (0·5 M), L-nuciferine (0·01 M in 0·165 M NaCl, pH 4·5) and NaCl (0·165 M).

The effects of L-nuciferine were determined on the excitatory responses induced in 9 Renshaw cells by equieffective ejecting currents of acetylcholine (5–30 nA), kainate (10–40 nA) and NMDA (10–30 nA), L-Nuciferine ejected with currents of 5–80 nA (mean 46 nA) for 2–6 min reduced the excitant effect of all three substances on 7 neurons. However, L-nuciferine appeared to depress responses to acetylcholine and kainate more than responses to NMDA. Thus, on 6 neurons, acetylcholine and kainate responses were reduced in parallel by 10–80% (mean 52%) whereas NMDA responses were only reduced by 20–40% (mean 35%). An example of this differential effect of

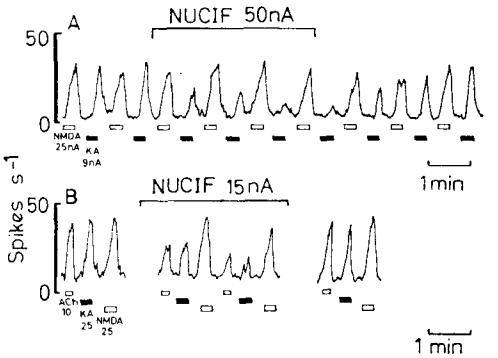

FIG. 1. Ratemeter record showing the effects of L-nuciferine (NUCIF) on the responses of (A) a dorsal horn neuron to kainate (KA 9 nA) and *N*-methyl-D-aspartate (NMDA 25 nA) and (B) a Renshaw cell to KA 25 nA, NMDA 25 nA and acetylcholine (ACh 10 nA). In A NUCIF 50 nA selectively and reversibly reduced responses induced by KA. In B, NUCIF 15 nA reversibly reduced responses to all excitants tested but ACh and KA induced responses were reduced considerably more than those induced by NMDA. The breaks in the lower record correspond to periods of approximately 2 min.

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L-nuciferine on one neuron is shown in the lower record of Fig. 1. Of the two remaining neurons tested, NMDA induced excitation was reduced more than acetylcholine and kainate induced excitation on one, whereas on the other only acetylcholine- and kainate-induced responses were reduced (both by 50%).

On all cells studied, the depressant action of L-nuciferine was accompanied by a marked reduction in spike amplitude and generally this effect preceded any reduction in sensitivity to neuronal excitants. Indeed, on one cell, the only effect observed with L-nuciferine was a reduction in spike amplitude. The depression of neuronal responses to excitants produced by L-nuciferine was not due to a reduction of spike amplitude below the level set on the window discriminator preceding the spike counter. On spontaneously active cells, L-nuciferine only slightly reduced the background firing in one case. All the effects of L-nuciferine reported above were reversible within 1–3 min of terminating the current ejecting the alkaloid.

L-Nuciferine had similar effects on amino acid-induced responses of dorsal horn neurons to those observed on Renshaw cells. Thus, 4–60 nA L-nuciferine for 2–5 min reduced the neuronal sensitivity to kainate (30–100%) more than that to NMDA (0–50%) in 5 neurons and produced a parallel reduction in kainate and NMDA excitatory responses in another neuron. In each case the reduction in neuronal sensitivity to excitants was preceded by a decrease in spike amplitude. The differential effect of L-nuciferine on responses to kainate and NMDA on one neuron is illustrated in the upper record of Fig. 1.

The present findings that L-nuciferine depresses responses of Renshaw cells to acetylcholine and the L-glutamate analogue, kainate, to a similar extent and that this effect is accompanied by a reduction in spike amplitude confirms the original results reported by Duggan et al (1973), and is compatible with a non-specific depressant action of L-nuciferine. However, the absence of effects of this alkaloid on acetylcholine responses in neurons of the ventrobasal thalamus (Ben-Ari & Kelly 1975) and pigeon optic tectum (Felix & Frangi 1977) are difficult to reconcile with the present results. If the effect of L-nuciferine on spike amplitude and neuronal sensitivity to excitants are two unrelated phenomena then it is possible that differences in acetylcholine receptor properties account for these discrepancies. Acetylcholine receptors on feline Renshaw cells are mainly nicotinic in nature (Curtis & Ryall 1966) whereas those in the ventrobasal thalamus have mixed nicotinic/muscarinic properties (Anderson & Curtis 1964). The acetylcholine receptors in the pigeon optic tectum have not been classified but the characteristics of the acetylcholine induced excitation in this area (Felix & Frangi 1977) is typical of that on other muscarinic receptors (Crawford & Curtis 1966; Krnjevic & Phillis 1963).

In contrast to the non-selectivity of L-nuciferine on responses to acetylcholine and kainate the alkaloid exhibited a differential effect on responses of spinal neurons to kainate and NMDA. Felix & Frangi (1977) and Polc & Haefely (1977) reported a similar differential effect of L-nuciferine on amino acid excitants and suggested that the alkaloid preferentially influenced L-glutamate receptors. However, the parallel reduction in acetylcholine and kainate sensitivity of Renshaw cells is not consistent with this suggestion. It is possible that on electrophoretic administration high, local concentrations of L-nuciferine are achieved near neuronal membranes which could explain the non-specific depressant action of L-nuciferine observed in these experiments, whereas following systemic injection (Polc & Haefely 1977) much lower concentrations of the alkaloid might prevail near glutamate-preferring receptors. It is unlikely, however, that the action of acetylcholine is mediated by releasing a glutamate-like amino acid from axon terminals on Renshaw cells or conversely, that kainate releases acetylcholine from such terminals since substances such as magnesium and HA-966 preferentially decrease responses evoked by NMDA and acetylcholine, whereas kainate induced responses were virtually unaffected (Davies & Watkins 1977; Biscoe et al 1978).

Thus, at present there is no satisfactory explanation for the differential effect of L-nuciferine on amino acid-induced excitation. Nevertheless, these results indicate that the specificity of this compound in the spinal cord when administered electrophoretically is not sufficient to recommend its use as an amino acid antagonist in the central nervous system.

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