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The Differential Sensitivity of Spinal Interneurones and Renshaw Cells to Kainate and N-Methyl-D-Aspartate

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Summary. With sensitivity to N-methyl-D-aspartate as the basis for comparison, spinal interneurones were relatively more sensitive than Renshaw cells to kainate, a conformationally restricted analogue of glutamate. These findings are consistent with proposed transmitter roles for L-glutamate and L-aspartate in the spinal cord.

Key words: Kainate — N-Methyl-D-aspartate — Spinal interneurones — Renshaw cells

Duggan (1974) found that Renshaw cells, which have not been shown to be monosynaptically excited by impulses in primary afferent fibres (Curtis and Ryall, 1966; Ryall and Piercey, 1971), are more sensitive to electrophoretically administered L-aspartate than to L-glutamate, whereas spinal dorsal horn interneurones that are monosynaptically activated from the periphery are more sensitive to L-glutamate than to L-aspartate. Taking into consideration problems associated with comparisons of the effectiveness of electrophoretically administered compounds, this differential sensitivity of the two populations of neurones supports the hypothesis that L-glutamate is an excitatory transmitter released by primary afferent fibres and L-aspartate an excitatory transmitter released by excitatory spinal interneurones. This hypothesis was previously based on neurochemical evidence of the spinal distribution of these amino acids (Graham, Shank, Werman and Aprison, 1967) and their known excitant actions on spinal neurones.

Although Renshaw cells were consistently more sensitive to L-aspartate than to L-glutamate, the mean glutamate: aspartate 'potency ratio' was 0.8, whilst that for interneurones was 1.2, the 'potency ratio' being the ratio of ejecting currents which produced equal and submaximal responses (Duggan, 1974). The molecules of glutamate and aspartate are flexible, and the relatively small potency difference may result from glutamate interacting with both glutamate and aspartate receptors. If glutamate interacts with glutamate receptors in an extended conformation, and with aspartate receptors in a folded conformation, then it is unlikely that aspartate, which has one less carbon atom in its chain than glutamate, could interact with such a glutamate receptor. If glutamate is 'non-specific' in that it interacts with both glutamate and aspartate receptors, then a greater relative difference in the sensitivities of Renshaw cells and interneurones would be expected with glutamate analogues that were more selective for the glutamate

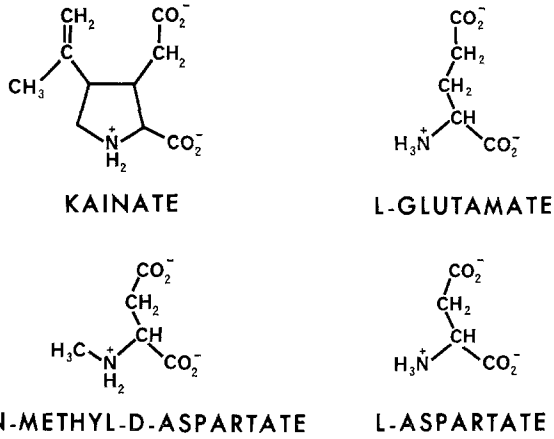

Fig. 1. Structural formulae of kainate, L-glutamate, N-methyl-D-aspartate and L-aspartate

receptor. Kainate, a potent amino acid excitant (Johnston, Curtis, Davies and McCulloch, 1974), is a conformationally restricted analogue of glutamic acid which is unlikely to occupy aspartate receptors, and hence should be more selective for the glutamate receptor than the parent molecule. On the other hand, N-methyl-D-aspartate (NMDA), which is comparable in potency to kainate, is probably too small a molecule to interact with the glutamate receptor (Johnston *et al.*, 1974). The structures of kainate, L-glutamate, NMDA and L-aspartate are illustrated in Fig. 1. This paper reports the relative sensitivities of spinal interneurones and Renshaw cells to kainate and NMDA as a further study in the elucidation of the transmitter roles of glutamic and aspartic acids.

Methods

The experiments were performed on Renshaw cells and interneurones of spinal lumbar segments of cats anaesthetised with pentobarbitone sodium (35 mgm/kgm intraperitoneal, supplemented when necessary) using previously described techniques (Curtis and Watkins, 1960). Renshaw cells were identified by the response to electrical stimulation of ventral roots, and interneurones by their activation following stimulation of the ipsilateral sural, tibial and common peroneal nerves. The central latencies of interneurones responding to impulses in hind limb afferents were determined by monitoring the incoming volley at the dorsal surface of the spinal cord.

Amino acids were administered electrophoretically as anions from seven barrel micropipettes, the 4 M NaCl-filled centre barrels of which were used to record extracellular action potentials. The outer barrels contained the following excitants: L-aspartate (1 M, pH adjusted to 8 with NaOH), DL-homocysteate (0.2 M, pH 8, NaOH), L-glutamate (1 M, pH 8, NaOH), N-methyl-D-aspartate (NMDA, 50 mM+200 mM NaCl, pH 8.3, NaOH), and kainate (5 mM+250 mM NaCl, pH 8.3, NaOH).

Both kainate and NMDA are potent neuronal excitants when administered as anions from 0.1 or 0.2 M solutions, excitation frequently occurring with mere reduction of the cationic retaining current. Greater accuracy would be expected in the comparison of the potency of two excitants when administered with comparable and readily measurable ejecting currents. Preliminary experiments established that approximately equal anionic ejecting currents (within the range 20 to 80 nA) were adequate to excite spinal neurones submaximally when solutions of NMDA and kainate were diluted with NaCl as above. It was assumed that the

fraction of the electrophoretic current ejecting the amino acid anions from dilute solutions in NaCl was 1/5 of that passed through the NMDA barrel and 1/51 of that through the kainate barrel.

To minimise any errors due to comparisons being made with different micropipettes an approximately equal number of Renshaw cells and interneurones were tested with any one micropipette. The relative potencies of kainate and NMDA were obtained by determining equipotent current ratios, the ratio of ejecting currents which produced equal, submaximal and steady-state firing rates. These ratios were corrected for the dilution factor of 10, and the reciprocal taken as a sensitivity ratio for any one neurone.

Results and Discussion

Seventeen Renshaw cells and 21 interneurones activated with a short central latency (<2 msec) from peripheral nerves were tested in 7 cats using 7 micropipettes. Responses to kainate and NMDA were generally slower in onset and recovery than those to glutamate or aspartate.

Table 1. Sensitivity ratios of spinal interneurones and Renshaw cells to kainate relative to NMDA

Cell Type	Classes of values of sensitivity ratio		
	>10	10	<10
Interneurone	16	4	1
Renshaw	0	0	17

The table contains numbers of cells with values of sensitivity ratio in classes.

A highly significant difference ($P<0.001$ for the chi-squared statistic of the contingency table and $P<0.001$ for Student's t statistic for the means and standard errors) was found in the relative sensitivities of the two groups of neurones (Table 1). All of the Renshaw cells that were tested had sensitivity ratios for kainate: NMDA that were less than 10, whilst only one of the sample of 21 interneurones had a ratio that was less than 10. The means and standard errors of the sensitivity ratios were 17.8 ± 2.2 for interneurones and 4.3 ± 0.5 for Renshaw cells, a 4—5 fold difference in the mean sensitivity ratios for the two groups of neurones. Thus, with sensitivity to NMDA as the basis of comparison, interneurones were relatively more sensitive to kainate than were Renshaw cells.

The observed differences in the relative sensitivities of interneurones and Renshaw cells to kainate and NMDA were considerably larger than those reported for L-glutamate and L-aspartate by Duggan (1974), who discussed in detail the various factors which might account for differences in the sensitivity of neurones to these two naturally occurring amino acids. These factors include the lack of exact information regarding micropipette characteristics, diffusion and inactivation of ejected excitants within the tissue and the location and specificity of amino acid receptors on particular neuronal membranes. In addition it is not possible to measure the relative intrinsic activity of kainate and NMDA at neuronal receptors. Kainate and NMDA are probably distributed differently in the tissue from glutamate and aspartate during electrophoretic administration. L-Glutamate and L-aspartate are taken up by high affinity transport systems in slices of spinal cord *in vitro*, and NMDA appears not to be a substrate for these systems (Balcar and

Johnston, 1972). Kainate is a weak competitive inhibitor (K_i 250 mM) of glutamate high affinity uptake by brain tissue slices (Stephanson and Johnston, unpublished) and thus can be only a relatively poor substrate for this transport system, although both kainic and NMDA acids may be taken up by other transport processes. The slow recovery of kainate and NMDA responses suggests that active uptake of these excitants contributes very little to the termination of their responses, although to some extent these slow time courses may reflect slow dissociation of amino acid-receptor complexes.

If it is assumed that kainate is more selective for the glutamate receptor than is glutamate, and that NMDA is too small a molecule to interact with the glutamate receptor, then the present results are consistent with the presence of both glutamate and aspartate receptors on spinal neurones, there being fewer glutamate receptors, in comparison with aspartate receptors, on Renshaw cells than on interneurones. The results thus provide additional support for the proposition that glutamate is the excitatory transmitter at primary afferent synapses in the spinal cord and that aspartate is the excitatory transmitter released by spinal excitatory interneurones. By using amino acid analogues to reveal such significant differences in the amino acid sensitivity of particular populations of neurones it may be possible to distinguish glutamate-mediated excitatory pathways from those mediated by aspartate in other regions of the central nervous system, although the use of specific antagonists of the excitants will be required to fully assess the synaptic role of these amino acids. A study of the interactions between such antagonists and kainate and NMDA may also provide an indication of the degree of specificity of all of these compounds in relation to amino acid receptors at excitatory synapses.

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