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The action of N-methyl-D-aspartic and kainic acids on motoneurones with emphasis on conductance changes

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Interactions of putative blockers with neuronal responses to excitatory amino acid analogues seem to indicate that there are two distinct agonist receptors (see review by Watkins, 1978): (a) a 'glutamate' receptor (with which the conformationally restricted analogue kainic acid preferentially interacts), (b) an 'aspartate' receptor (with which N-methyl-D-aspartate (NMDA) preferentially interacts). We have investigated the membrane conductance (G_M) changes underlying the response to these analogues.

L-glutamate (1 M), L-aspartate (1 M), kainate (20 mM), NMDA (0.2 M) and D-homocysteate (0.2 M) (all *ca* pH 8, ejected as anions) were applied iontophoretically from the outer barrels of a coaxial electrode to cat lumbar motoneurones (the screened central barrel recording intracellularly).

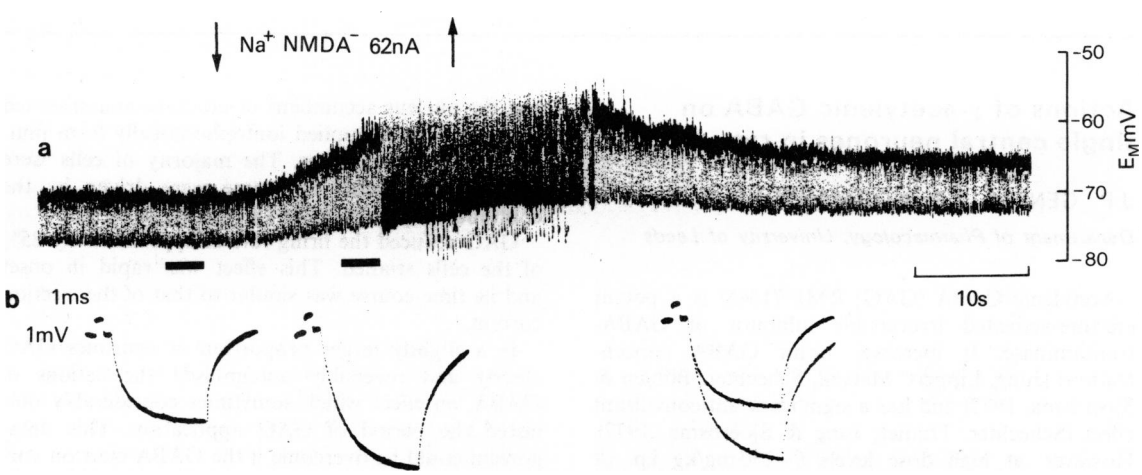

Figure 1 Depolarizing response of a lumbar motoneurone in a decerebrated cat to a current balanced iontophoretic application of N-methyl-D-aspartate (NMDA). The membrane potential record (E_M) is modulated by conductance measuring pulses (−3 nA, 12 ms constant current pulses injected through the screened recording electrode) and the AHPs of NMDA evoked firing (wide, dark band). High frequency repetitive firing resulted when NMDA had depolarized the cell by 9 mV and continued for 12 s after the ejecting current was turned off. The membrane potential subsequently recovered. Conductance measuring pulses below the potential record were averaged during the periods shown by the black bars (30 samples) and are shown superimposed on the right. Just before the NMDA induced firing, G_M had decreased to 62% of the control value.

The responses to glutamate and aspartate were very similar—a rapidly plateauing depolarization with little or no change in G_M with moderate doses. NMDA gave a 'triangular' shaped response accompanied by a very large decrease in G_M (Figure 1), even larger than that seen with D-homocysteate (Lambert, Flatman & Engberg, 1978). Long applications of NMDA evoked persistent firing.

Kainate was extremely potent—ejecting currents of 5-20 nA routinely evoked depolarizations of 30–40 mV. During the slow climbing phase of the response there was little change in G_M (an increase in G_M when allowing for anomalous rectification). The response would continue to climb through a short phase of firing and finally arrive at a plateau—usually at a membrane potential of −20–−30 mV. At the plateau G_M was immeasurably large. Recovery was slow and seldom complete (except with *very* small doses). Motoneurone axons were essentially unresponsive to kainate.

Responses to glutamate and aspartate are qualitatively very similar, while that to NMDA is so dissimilar to both as to question that aspartate and NMDA act on the same receptor (even allowing for the absence of NMDA uptake). Moreover, the phenomenal potency, slow onset of action, large G_M increase and irreversibility characteristic of kainate depolarizations poses the questions whether kainate interacts with a 'glutamate' or an 'aspartate' receptor.

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Do primary afferent terminals have acidic amino acid receptors?

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Recordings from dorsal roots of rat isolated spinal cords suggest that afferent terminals are depolarized not only by GABA and other neutral amino acids but also by a range of excitatory amino acids (Evans 1978). However it is possible that the dorsal root depolarizations produced by acidic amino acids are not generated directly via presynaptic receptors for these substances but indirectly via release of some depolarizing substance such as potassium or a transmitter into extracellular space. Provided the rate of reuptake or synthesis of such a substance was slower than the release, then such indirect responses would be expected to fade during prolonged superfusion of excitant (as the released substance is flushed away) and subsequent applications of excitant should be reduced or abolished.

Prolonged treatment (30–120 min) of rat isolated hemicords with the potent excitant N-methyl-D-aspartate (NMDA, 25–200 μM), had an effect consistent with the above possibility. Such applications of NMDA produced depolarization recorded in dorsal roots which faded back to the resting polarity with a half time of 5–10 minutes. However, the concomitant depolarization recorded from ventral roots showed only a partial fade, with a time course similar to that shown by dorsal roots, and ventral roots remained depolarized at equilibrium. This residual ventral root depolarization was maintained for several hours, suggesting that motoneurones are depolarized directly by NMDA.

Similar effects were observed with hemisected frog spinal cords, and in both species, after prolonged NMDA treatment, no depolarizing responses could be evoked by NMDA at a thousand times the usual threshold level (1 μM). In contrast, kainate still depolarized dorsal (but not ventral) root fibres of frog or rat isolated hemicords in the continued presence of NMDA, although NMDA treated tissues were at least 10 times less sensitive to kainate than controls.

Dorsal root sensitivity to GABA was not significantly altered by NMDA treatment, and in frog preparations during such treatment GABA responses were similar to those of dorsal roots attached to control hemicords rather than to those of isolated dorsal roots, which had a much longer time course. This indicates that afferent terminals are unlikely to have been damaged by the NMDA treatment.

These effects suggest either that presynaptic terminals desensitize to NMDA and only partially to kainate or that these terminals have kainate receptors but not NMDA receptors. If the latter possibility is correct, it would indicate that depolarizations of affer-