Selective antagonism by Mg^{2+} of amino acid-induced depolarization of spinal neurones[1]

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Summary. In the isolated frog or rat spinal cord, low concentrations of Mg^{2+} (0.5–1.00 mM) markedly depress, in a substantially Ca^{2+}-independent manner, ventral root depolarizations produced by dorsal root stimulation and by certain amino acids (e. g. N-methyl-D-aspartate and L-homocysteate) but do not depress depolarizations produced by other excitatory amino acids (e. g. kainate and quisqualate). L-Aspartate-induced depolarizations are more sensitive to Mg^{2+} then are L-glutamate-induced depolarizations.

Not only L-glutamate and L-aspartate, but also several related amino acids must be considered as possible transmitters at excitatory synapses in the vertebrate central nervous system[2,3]. These include L-cysteine sulphinate[4] and L-cysteate[5], which are known to be present in mammalian central nervous tissue, and L-homocysteine sulphinate and L-homocysteate which have been shown to be formed in liver[6,7] and could conceivably be present in central nervous tissue as metabolites of homocysteine or methionine. Different receptors and/or receptor mechanisms for the action of different amino acid excitants have recently been recognized in invertebrates[8–10] and there have been indications that a similar situation may obtain in vertebrates. Thus, in the mammalian central nervous system, separate receptors have been postulated for L-glutamate and L-aspartate on the basis of the differential sensitivities of discrete populations of spinal neurones to the 2 putative transmitters[11], and especially to the more conformationally restricted analogues, kainate and N-methyl-D-aspartate[12]. Also, Engberg, Flatman and Lambert[13] recently demonstrated that DL-homocysteate caused a different membrane conductance change in cat motoneurones from that caused by L-glutamate[14–16], and we showed that the effects of changes in $[Na^+]$ or $[K^+]$ on L-homocysteate-induced depolarizations of frog or immature rat spinal motoneurones were clearly different from the effects on depolarizations induced by L-glutamate and other amino acids[17]. We now report that low concentrations of Mg^{2+} selectively antagonize amino acid-induced depolarization of motoneurones, the effects being greatest on L-homocysteate and N-methyl-D-aspartate, and least on quisqualate and kainate, while L-asparate responses were significantly more sensitive than L-glutamate responses.

Material and methods. Experiments were conducted mainly on the isolated hemisected spinal cord of Rana pipiens and R. temporaria as previously described[18,19]. The standard superfusion medium contained (mM): NaCl, 111; KCl, 2; $CaCl_2$, 2; tris, 10; glucose, 12, adjusted to pH 7.5 with HCl. The temperature was maintained at 12.5 ± 0.5 °C and the flow rate was 1.5 ml per min. Procaine (10^{-3} M) or tetrodotoxin (10^{-6} M) were included in the medium where it was desired to eliminate indirect actions of amino acids on motoneurones arising by excitation of interneurones[18,19]. In some experiments, the isolated hemisected spinal cord of immature rats (4–8 days old)[20] was used. In this case, the composition of the medium was as follows (mM): NaCl, 118; KCl, 2: $NaHCO_3$, 24; $CaCl_2$, 2.5; glucose, 12. This medium was gassed with 95% O_2, 5% CO_2 and the temperature was maintained at 20 ± 0.5°; the flow rate was 0.8 ml per min. Similar results were obtained with either of these tissues.

Results and discussion. Figure 1 shows the effect of the addition of 0.5 mM Mg^{2+} to the superfusion medium on the ventral root potential evoked by dorsal root stimulation (DR-VRP) and on the depolarizations produced by

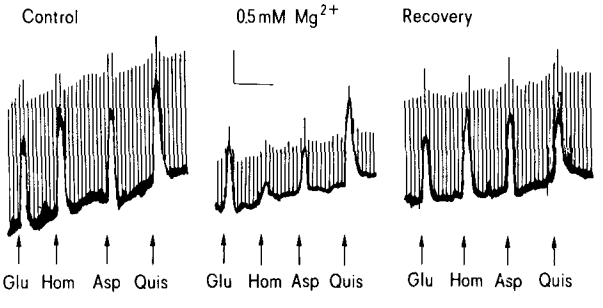

Fig. 1. Effect of 0.5 mM Mg^{2+} on ventral root depolarizations evoked by dorsal root stimulation (DR-VRP) and by different excitatory amino acids on the isolated frog spinal cord. The dorsal root was stimulated at 2/min (0.05 msec, 30 V). Concentrations of the amino acids were adjusted to produce approximately equal ventral root depolarizations in the control series. The central record begins 35 min after changeover to superfusion medium containing 0.5 mM $MgSO_4$. The recovery series began 72 min after return to normal (Mg^{2+}-free) medium. GLU = L-glutamate, 1000 µM; HOM = L-homocysteate, 35 µM; ASP = L-aspartate, 2000 µM; QUIS = quisqualate, 2,5 µM. All amino acids were applied for 40 sec. Calibration: 1 mV; 4 min.

1 Acknowledgment. We thank D. J. Oakes for skilled technica assistance. This work was supported by the Medical Research Council.

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Depression of amino acid-induced ventral root depolarizations by Mg^{2+} (1mM)

Amino Acid	Per cent control
Kainate	99.8 ± 4.6 (5)
Quisqualate	92.9, 100, 100*
L-Glutamate	85.4 ± 4.3 (6)
L-Aspartate	71.8 ± 4.4 (4)
D-Homocysteate	71.1 ± 8.8 (3)
L-Cysteate	59.4 ± 6.3 (3)
L-Cysteine sulphinate	47.1, 63.8
L-Homocysteine sulphinate	50.0, 53.7
L-Homocysteate	32.3 ± 2.2 (8)
D-Aspartate	32.7, 29.3
D-Glutamate	30.9 ± 1.4 (3)
N-Methyl-D-Aspartate	12.7 ± 2.9 (4)

The experiments were conducted in superfusion medium containing 1 mM procaine. Amino acids were tested in groups of 3 or 4 on each spinal cord (12 preparations in all) and the concentration of each amino acid was adjusted so that all gave equal depolarizations of between 1 and 3 mV under control conditions. The period of contact of the amino acid-containing solutions with the spinal cord was 40 sec. The effect of Mg^{2+} was determined by the addition of 1 mM $MgSO_4$ to the superfusion medium, amino acid responses in this medium being measured 2 or 3 times during a 50–80-min-period beginning 10 min after the changeover. Responses were generally stable over the whole period, but where slight time-dependent changes were observed, responses were all corrected to a single time point between 20 and 30 min after changeover to the Mg^{2+}-containing medium. Responses are expressed as percent of the control responses obtained immediately before the addition of Mg^{2+}. Figures are means ± SEM with the number of observations, on different preparations, given in parenthesis; where less than 3 preparations were used, the individual results are given. *4 mM $MgSO_4$.

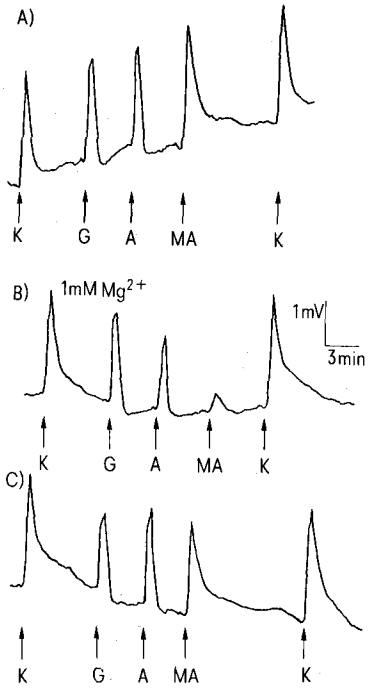

Fig. 2. Effect of 1 mM Mg^{2+} on ventral root depolarizations induced by different amino acids in the procaine-blocked frog spinal cord. *A* Control series, showing matched responses. *B* Recorded 10 min after changeover to superfusion medium containing 1 mM $MgSO_4$. *C* Recorded 16 min after return to normal (Mg^{2+}-free) medium. All media contained 1 mM procaine. K = kainate, 10 μM; G = L-glutamate, 800 μM; A = L-aspartate, 1200 μM; MA = N-methyl-D-aspartate, 70 μM. The period of contact of the amino acid-containing solutions with the spinal cords was 40 sec.

L-glutamate, L-aspartate, L-homocysteate and quisqualate in the frog spinal cord. The amplitude of the DR-VRP was reversibly reduced by about 60% in the presence of Mg^{2+} and the responses to L-homocysteate and L-aspartate by approximately 80% and 50% respectively. Although there was also an apparent reduction in the responses to both L-glutamate and quisqualate, these effects were not reversed by the removal of Mg^{2+} from the medium.

Similar effects of Mg^{2+} on amino acid responses were also observed when procaine or tetrodotoxin were present in the medium, though in these cases the depressions of the responses were somewhat smaller than in the absence of blocking agents. These reduced effects would be expected since part of the depression of the amino acid responses produced by Mg^{2+} in the unblocked preparations can be assumed to be due to decreases in the amino acid induced activity of excitatory synaptic pathways terminating on motoneurones. The antagonistic effects of Mg^{2+} on the ventral root responses produced by a range of amino acid excitants on the procaine-blocked frog spinal cord are shown in the table.

The table indicates that the responses produced by certain amino acids, including L-glutamate, kainate and quisqualate are relatively insensitive to the presence of Mg^{2+}, whereas the responses produced by N-methyl-D-aspartate (especially), L-homocysteate, D-aspartate and D-glutamate are markedly depressed by low concentrations of these ions. The responses of a further group of amino acids, including L-aspartate, are depressed to an intermediate degree by Mg^{2+}. A clear difference between the effects of Mg^{2+} on L-glutamate and L-aspartate induced responses was invariably observed in direct comparisons on the same preparations ($p < 0.001$, paired t-test, 12 direct comparisons on 8 preparations, with either 0.5 or 1 mM Mg^{2+}). Figure 2 illustrates the differential action of 1 mM Mg^{2+} on the responses produced by L-glutamate, L-aspartate, kainate and N-methyl-D-aspartate.

The same concentrations of Mg^{2+} as those which antagonized amino acid-induced responses caused no detectable shift in the base-line level of ventral root polarization, suggesting that the metal ions had little effect on the resting membrane potential of motoneurones. Possible explanations for the antagonism by Mg^{2+} of the depolarizing action of particular amino acids include: a) facilitation of mechanisms which remove the amino acids from their site of action, b) blockade of a presynaptic action of the amino acids (e.g. the release of excitatory transmitters or K^+ from synaptic terminals), and c) direct depression of the sensitivity of the motoneuronal membrane to the amino acids. The first possibility may be regarded as unlikely, since the relative sensitivity of the amino acid induced responses to Mg^{2+}, unlike those to Na^+ and K^+[17], do not show any correlation with the known characteristics of amino acid uptake. Thus, substances which produce responses of widely disparate sensitivity to Mg^{2+} (table) may have similar uptake properties. For example, L-glutamate and D-aspartate are actively accumulated by central nervous tissue[21–23], while the available evidence suggests that kainate[24] and N-methyl-D-aspartate[25] are not. The second possibility cannot

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readily be excluded but it may be relevant in this respect that increasing the medium [Ca^{2+}], which reverses the depressant effect of Mg^{2+} on the synaptic release of transmitters from cholinergic[26] and probably also from adrenergic[27] terminals, did not reverse the effects of Mg^{2+} on the amino acid-induced responses of ventral roots. Indeed, increases in [Ca^{2+}] also produced depressant effects on these responses which were additive with, but considerably weaker than those produced by Mg^{2+}.

The third possibility would imply the involvement of different types of post-synaptic receptors for excitatory amino acids. In this case, kainate and quisqualate might be considered to act almost exclusively on Mg^{2+}-insensitive receptors, and N-methyl-D-aspartate on Mg^{2+}-sensitive receptors. Other amino acids may be presumed to act to a greater or lesser extent on both types of receptors. Such an hypothesis would accord with the different actions of DL-homocysteate and L-glutamate on cat spinal motoneurones[13], and with the differential sensitivity of different groups of cat spinal neurones to L-glutamate and L-aspartate[11] and especially to kainate and N-methyl-D-aspartate[12].

It may be significant that the DR-VRP is markedly attenuated by such low concentrations of Mg^{2+} in the presence of normal [Ca^{2+}]. We have shown that this depression is only partially reversed by increasing the [Ca^{2+}] of the medium, as also noted by Katz and Miledi[28]. Thus, not all of the depression of the DR-VRP by Mg^{2+} may be due to inhibition of transmitter release[26]. This raises the possibility that Mg^{2+}-sensitive transmitter receptor sites may be involved in the generation of the DR-VRP.

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Effect of lanthanons on substrate-induced difference spectra in rat liver microsomes

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Summary. Intravenously administered light lanthanons change spectral interactions in rat liver not only by decreasing the concentration of cytochrome P-450, but they also cause a qualitative change in the cytochrome P–450 molecule or its microenvironment.

The lanthanons are divided into light and heavy elements, having different biological effects. Intravenously administered light lanthanons (Lanthanum–Samarium) cause fatty infiltration of the liver[2] and impairment of the drug metabolism[3]. The heavy lanthanons (Europium–Lutetium) cause only focal necroses of the liver without changes in the lipid concentration[4] or the metabolic activity[5]. Light lanthanons have been shown to decrease the amount of cytochrome P-450 (Cyt. P-450) to about 50% of its initial value, whereas the decrease caused by the heavy lanthanon, Erbium, is only 10%[5]. The addition of various substances, such as hexobarbital or aniline, to liver microsomes induces difference spectra which are considered to be indicative of the binding to the Cyt. P-450, the terminal oxidase of the microsomal electron transport chain[6,7].

The aim of the first part of this study was to compare the effects of a light lanthanon, Cerium (Ce), and a heavy one, Erbium (Er), on the substrate binding capacity of the Cyt. P-450 in the microsomal fraction of rat liver. Possible changes in spectral interactions may indicate qualitative differences in the function of the decreased Cyt. P-450 caused by lanthanons. The second part of our study was based on the report of Lehmann et al.[8] who found differ-

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Table 1. The liver weights and the amounts of Cyt. P-450 and b_5 in variously treated groups of male rats ($\pm$ SD)

	Liver weight (g)	Rel. liver weight (g)	Cyt. P-450 nmoles/mg prot.	Cyt. b_5 nmoles/mg prot.
Group I Controls	9.03 $\pm$ 1.18	4.47 $\pm$ 0.12	0.556 $\pm$ 0.150	0.346 $\pm$ 0.141
Group II Ce	12.49 $\pm$ 0.80*	5.36 $\pm$ 0.29*	0.348 $\pm$ 0.042*	0.182 $\pm$ 0.044*
Group III Er	9.19 $\pm$ 1.11	4.19 $\pm$ 0.37	0.520 $\pm$ 0.138	0.386 $\pm$ 0.061

*$p < 0.01$