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Failure of methyltetrahydrofolate to mimic or antagonize kainate-induced responses of spinal or trigeminal neurons

R. H. Evans, R. G. Hill, T. S. Salt, D. A. S. Smith, *Department of Pharmacology, Medical School, University Walk, Bristol, BS8 1TD, U.K.*

Neuropharmacological interest in pteroylglutamate compounds stems from the initial observation that serum folate levels were reduced in epileptic patients undergoing anticonvulsant drug therapy (Reynolds 1972). Subsequently, it has been shown that convulsions can be induced in experimental animals following intraventricular or intracortical application of folate or folinate (Hommes & Obbens 1972; Spector 1971). These compounds have been shown also to excite cortical neurons in the cat directly following microelectrophoretic application (Davies & Watkins 1973; Hill et al 1974).

More recently methyltetrahydrofolate (MTHF) has been reported to be a potent displacer of the neuroexcitant amino acid kainate from membranes prepared from rat brain, suggesting that the excitant action of MTHF is caused by an action at kainate receptors (Ruck et al 1980). In support of this suggestion Olney et al (1981) have reported that folate compounds including MTHF have a kainate-like neurotoxic action.

In the present paper we report that MTHF, applied either microelectrophoretically to single neurons in the rat brain stem or in the bathing medium of isolated spinal cord preparations, failed to mimic or antagonize excitatory responses induced by kainate.

Recordings were made of the firing rate of neurons in the caudal trigeminal nucleus of urethane anaesthetized rats via the 4 M NaCl filled centre barrel of multibarrelled ionophoretic electrodes. Kainate was applied from barrels which contained 15 mM kainate (Sigma) dissolved in 150 mM NaCl. MTHF (Sigma Grade II sodium salt) was applied from barrels containing 100 mM MTHF (aqueous solution pH 7·0). D.C. recordings of motoneuron polarity were made from ventral roots of hemisected spinal cord preparations taken from 4–8 day old rats (Otsuka & Konishi 1974).

MTHF in concentrations up to 500 μM had no depressant action on dorsal root evoked tranmission or kainate (2–8 μM) induced depolarizations recorded in ventral roots of three rat spinal cord preparations. Fig. 1a illustrates the failure of MTHF (500 μM) to depress dorsal root evoked electrical activity recorded from a ventral root of a rat spinal cord preparation. It can be seen that the presumed glutamate antagonist 2-amino-4-phosphonobutyrate (100 μM) White et al 1977) produced a marked depression of this synaptic response. A similar lack of effect of 500 μM MTHF was observed also in a frog spinal cord preparation (not illustrated). Fig. 1b shows a recording from the same preparation following blockade of transmission with tetrodotoxin (Evans & Watkins 1978). It can be seen that MTHF (500 μM) neither mimicked nor antagonized the depolarizations induced by application of 2 μM kainate.

In rat caudal trigeminal nucleus MTHF was found to excite 9 of the 14 neurons to which it was applied with currents in the range of 50 to 250 nA, it had no effect on 4 neurons and depressed 1 neuron. In spite of the high currents used the excitant action of MTHF was weak. In contrast, the application of kainate (range 15–75 nA) produced marked excitation of all of 105 neurons tested. On two neurons MTHF was tested for its ability to antagonize the excitation produced by ionophoretic application of kainate and was ineffective in both cases.

Information on the relative electrophoretic mobilities of MTHF and kainate is not available. However, in the above experiments kainate was diluted tenfold with chloride and applied at a quarter of the currents used to apply MTHF. Thus for the two agents to be equipotent a difference in transport number of a least fortyfold would be required.

The either very weak or absent neuroexcitant action of MTHF when tested on trigeminal and spinal preparations respectively and the failure of MTHF to mimic or antagonize the neuroexcitant action of kainate in either preparation does not support the suggestion, based on displacement of kainate from rat cerebellar membranes that MTHF is an endogenous ligand for the kainate receptor (Ruck et al 1980). These workers have reported that kainate is about 10 times more potent than MTHF and MTHF is itself some 30 times more potent than L-glutamate in displacing labelled kainate. Kainate is approximately 100–400 times more potent than L-glutamate as a neuroexcitant (Biscoe et al 1976; Evans 1978) which approximates to the relative potencies of these two ligands in the above binding study. However, in the present experiments the threshold concentration for depolarizations evoked by kainate was less than 0·5 μM whereas MTHF was inactive at 500 μM indicating a difference of at least a thousand fold between the neuroexcitant potencies of MTHF and kainate. The lack of effect of MTHF could be attributed to the presence of systems designed to remove this endogenous ligand from the extracellular space. However, despite the presence, in central nervous tissue, of active uptake systems for L-glutamate the threshold concentration for the excitatory action of this endogenous amino acid on isolated spinal cord preparations is around 50 μM (Evans & Watkins 1975).

It is possible that methodological differences may underlie the inconsistency between the present observations and

* Correspondence.

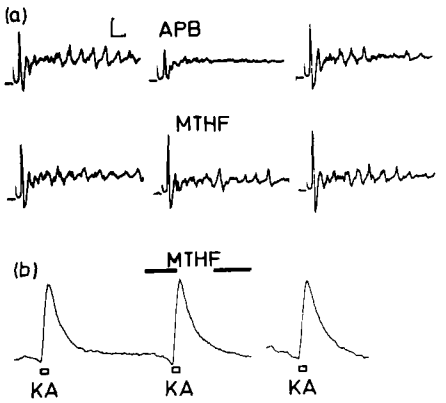

FIG. 1(a). Oscillographic records of dorsal root (supramaximal stimuli 2 min^{-1} 0·5 ms duration) evoked potentials recorded from the ventral root (L5) of a hemisected spinal cord preparation from a 5 day old rat. Top row of traces shows depressant effect of 100 μM 2-amino-4-phosphonobutyrate (APB). Bottom row shows lack of effect of 500 μM methyl-tetrahydrofolate (MTHF). Left hand traces 3 min before and centre traces 5 min following introduction of test compound. Right hand traces 15 min following removal of test compounds from the bathing medium. (b) Pen recording of ventral root polarity, depolarization of motoneurons upwards. Same preparation as in (a) blocked with tetrodotoxin (0·1 μM). 500 μM MTHF and 2 μM kainate applied as indicated. It can be seen that MTHF did not either induce depolarization itself or antagonize the kainate-induced depolarization. Calibration 2·5 mV, 20 ms in (a); 0·25 mV, 4 min in (b).

those of Loots et al (1981), who observed both MTHF-induced excitation and depression in frog spinal cord preparations. However, until the latter work is published it will not be possible to comment on what these differences might be.

The results of lesion studies suggest that MTHF and kainate act at different sites. For instance MTHF did not damage neurons at the injection site, as does kainate, but mimicked only the kainate-induced indirect seizure-mediated pattern of brain damage (Olney et al 1981). Since diazepam, which is an ineffective antagonist of kainate (Evans et al 1977), protects against seizure-mediated damage (Ben-Ari et al 1980) it would seem that such damage need not necessarily be a consequence of kainate receptor activation.

Structure-activity considerations alone would not suggest an effect of MTHF at excitatory amino acid receptors since linkage of the α-amino group of glutamate with the carboxylate group of the *p*-aminobenzoate moiety of pteroylglutamate compounds results in loss of basicity of the α-amino group. Furthermore, the pteroyl moiety represents a very large substituent group on the α-amino nitrogen atom. Both these factors would be predicted to result in a loss of neuroexcitant activity (Curtis et al 1960; Curtis & Watkins 1963).

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