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SHORT COMMUNICATIONS

Facilitatory and direct excitatory effects of folate and folinate on single neurones of cat cerebral cortex

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IT IS well established that serum folate levels are reduced in epileptic patients undergoing anticonvulsant drug therapy[1] but whether correction of the folate deficiency increases the frequency and severity of the epileptic episodes is controversial.[2–4] There is some evidence that folate therapy itself may result in reduced serum levels of anticonvulsant drugs which could account for this controversy.[5] On the other hand, animal studies have shown that convulsive episodes can be induced by systemic and intracortical injection of sodium folate and intraventricular injection of calcium folinate (N^5-formyl-5,6,7,8-tetrahydrofolate).[6,7] It has also been shown that interfering with folate metabolism may decrease the convulsive action of leptazol.[8] These findings suggest the possibility of a more direct involvement of folate and its metabolites in neuronal excitability. Hence, a preliminary investigation has been made of the direct effects of folate and folinate on single neurones in the cat cerebral cortex using standard microelectrophoretic techniques.

The experiments were performed on pericruciate neurones of cats lightly anaesthetized with N_2O–halothane as described elsewhere.[9] Seven-barrel glass micropipettes were used, one barrel of which contained 2 M NaCl for extracellularly recording the responses of neurones, and the other barrels contained various combinations of the solutions from which the electrophoretic applications were to be made. The latter comprised Na L-glutamate (200 mM, pH 8), Na folate (200 mM, pH 8), Na folinate (200 mM, pH 8), GABA 0·5 HCl (200 mM, pH 3·5) and acetylcholine chloride (200 mM, pH 4). Spike responses were monitored continuously on an oscilloscope, counted with a ratemeter and displayed on a pen-recorder trace.

The effects of iontophoretically applied folate and folinate were studied on 25 pericruciate neurones which were either firing spontaneously or excited by a background application of L-glutamate (5–30 nA) or acetylcholine (25–50 nA). Folate and folinate ejected with currents of 15–50 nA both caused a substantial increase (25–100 per cent) in the firing rate of all the neurones tested. Occasionally, the excitation was so marked that a depolarization block resulted. The typical effect of folinate on a cell excited by regular applications of L-glutamate is illustrated in Fig. 1. In all tests folinate was more effective than folate in increasing the firing rate of spontaneously active and chemically excited neurones. Thus, on five spontaneously firing cells where direct comparisons were made folinate was 0·25–0·5 times as effective an excitant as L-glutamate (on a current basis) and folate about half as effective as folinate.

When tested on quiescent neurones which could be readily excited by L-glutamate, folate and folinate exhibited only very weak excitatory effects. Thus, folinate (50–100 nA) caused a low frequency firing in three out of four tests and folate (100 nA) had a similar effect in one out of sefi·vstet

The effects of the pteridines were also determined on seven neurones inhibited by GABA. These neurones were excited by L-glutamate or acetylcholine and suppression of firing was usually achieved

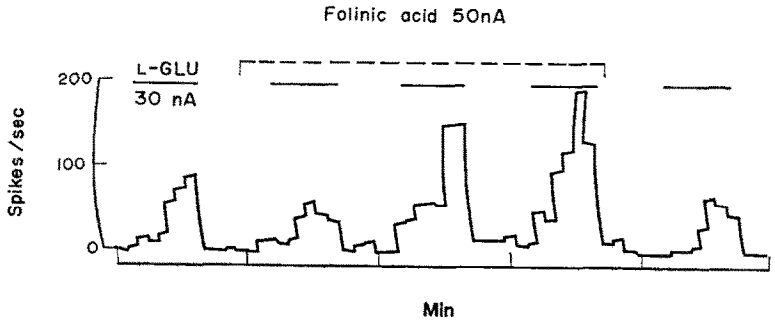

FIG. 1. Effect of folinic acid on the firing rate of a pericruciate neurone excited by L-glutamate. The pen-recorded histogram shows the number of spikes per 5 sec interval caused by the regular administration of L-glutamate pulses (30 nA, 30 sec). Co-administration of folinic acid (50 nA, 165 sec caused a marked increase in the firing rate.

by ejection of GABA with currents of 10–20 nA. Folinate, ejected with currents of 15–25 nA, reduced the depressant action of GABA by 40–80 per cent on four out of five cells tested and folate (25–50 nA) had a similar effect in three out of four tests. However, the reduction of the depressant effect of GABA was only seen once in the absence of a marked increase in the background firing rate of the cells.

The results of this investigation indicate that folate and folinate can directly influence neuronal excitability in the central nervous system. However, the mechanism of these actions are not known. The excitation of quiescent neurones suggests the possibility of a direct action on the neuronal membrane leading to depolarization of the cells. This could arise through the action of the glutamate moiety of the compounds on membrane receptors which interact with excitatory amino acids. However, *N*-acylated amino acids have usually been inactive in investigations of the structure-activity relations of this type of action.[10] It is possible that enzymic hydrolysis of the compounds occurred in the extracellular fluid, and that the excitatory effects were due to the free glutamate so formed. Another possibility, that free glutamate may have been present in the original electrophoretic solutions used, was excluded by paper chromatography and high voltage paper electrophoresis. The significance of the antagonism of GABA by the two pteridines is difficult to assess, since the background rate was usually increased in these tests, which alone might be expected to decrease the effectiveness of the inhibitory amino acid. Nevertheless, the possibility that a blockade of GABA receptors contributed to the observed excitatory effects of the compounds cannot be ruled out.

Whatever the mechanisms involved, it is clear that both folate and folinate increase the firing rate of active neurones in the pericruciate cortex. This finding is probably related to the observation that intracortically administered folate causes convulsions in normal animals and activates a cobalt-induced epileptic focus.[6] The concentrations of the pteridines obtained in the extracellular medium by microelectrophoresis are likely to be of the order of 10^{-3} M[11,12] which is similar to that injected intracortically.[6] However, such concentrations are several orders of magnitude in excess of that which would be expected to be obtained in the extracellular space around neurones as a result of the usual therapeutic dose administered. Thus, while it is possible that neurones within epileptogenic foci are more sensitive to folate and folinate because of their high excitability, it is not known whether therapeutic doses of folate used could exert such an effect in epileptic patients.

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