ANTAGONISM BY FOLIC ACID OF PRESYNAPTIC INHIBITION IN THE RAT CUNEATE NUCLEUS

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Folic acid applied topically in solution to the rat cuneate nucleus reduced presynaptic inhibition produced by peripheral stimulation. This resembles the action of picrotoxin in the same animals and it is possible that these two convulsants are acting in a similar manner by blockade of the receptors for the presynaptic inhibitory transmitter.

Introduction

Folic acid produces convulsions when injected into the brains of rats (Hommes & Obbens, 1972; Hill, Miller, Straughan & Webster, 1974) but the mechanism of this convulsant action is not clear. The presynaptic inhibitory process of the cuneate nucleus is blocked by a number of other convulsant substances (Banna & Jabbur, 1969; Davidson & Southwick, 1971) and accordingly we have examined the effect of topical application of folic acid solutions upon this inhibition.

Methods

Experiments were performed on six adult piebald Lister rats anaesthetized with urethane (0.6 ml of a 25% w/v solution per 100 g of rat). The head was fixed in a stereotaxic frame and the cuneate nucleus was exposed by removal of the atlanto-occipital membrane and overlying tissues.

Bipolar needle electrodes were inserted into the central pad of one forepaw and rectangular pulses (3 to 5 V, 0.2 ms, 0.5 Hz) were delivered continuously from a Grass SD9 stimulator. A glass microelectrode (tip size c. 6 μm) containing 3 M NaCl was advanced into the cuneate nucleus ipsilateral to the paw being stimulated, until an evoked potential of maximal size was recorded. Using a small laboratory computer (Biomac 1000, Datalabs Ltd), 32 potentials were averaged over a sweep time of 160 ms at regular intervals of 2 or 3 min and each complete average was written out on an X-Y recorder. Potentials were analysed by measuring the N- and P-wave components as described by Banna & Jabbur (1969) and the amplitude of the P-wave was taken to be representative of the depolarization of primary afferent fibres associated with presynaptic inhibition. Drugs were applied to the surface of the cuneate nucleus from a fine polyethylene cannula as solutions in physiological saline at neutral pH. Folic acid (prepared as the sodium salt with NaOH) was used at concentrations from 0.1 to 1.0% w/v (c. 1 to 10 mM) and picrotoxin was used at 0.2% w/v (c. 3 mM).

The preparation was stable for many hours and it was therefore possible to perform more than one determination on each animal, always allowing recovery of the evoked potential to its control amplitude before applying a second drug.

Results

In all experiments solutions of folate stronger than 0.5% w/v reduced the amplitude of the P-wave, although weaker solutions were frequently ineffective. A 1.0% w/v solution produced twitching of the vibrissae and myoclonic jerks at a time when a maximal depression of the P-wave was seen and the convulsive activity was of sufficient severity to necessitate termination of the experiment. In five experiments with a 0.5% w/v solution, where good recovery of the control potential was achieved, the mean reduction in the P-wave amplitude was 76% (±11.4).

One of these experiments is illustrated in Figure 1. To the left of the figure are shown individual averaged potentials, selected at various times during the experiment, clearly showing the reduction of the P-wave. On the right, the measurements of P-wave made throughout the experiment are plotted sequentially, the letters over the graph indicating the individual potentials shown on the left. When it appeared that a maximum effect had been achieved the cuneate area was washed with saline and a recovery period allowed. Although not shown in this figure, a

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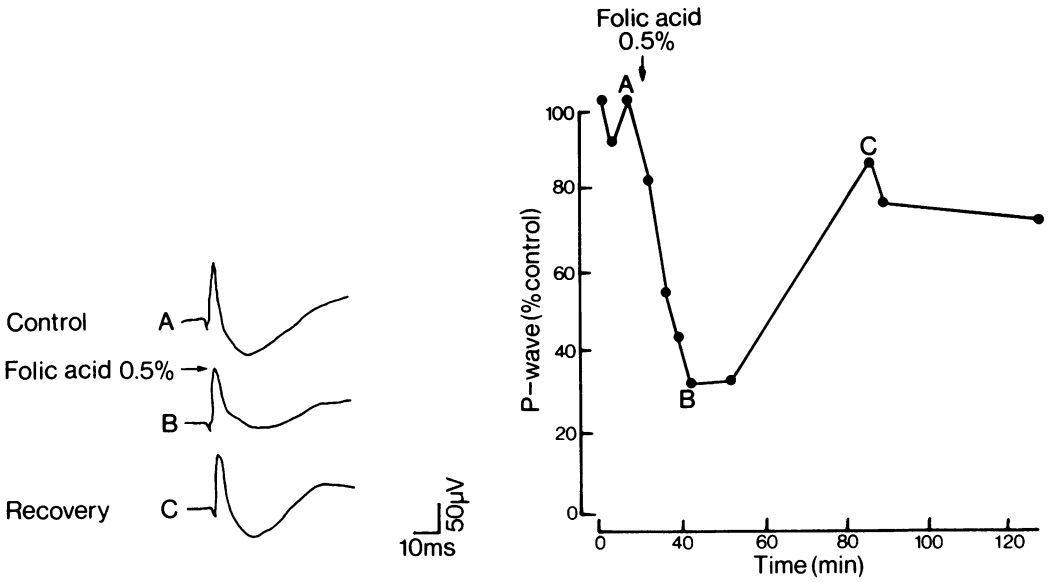

Fig. 1 To the left of the figure are shown examples of individual averaged potentials taken at various times during the experiment, negativity shown as an upward deflection. Ten ms after the start of the sweep the stimulus was triggered (small positive artifact), producing the fast N-wave and the more prolonged P-wave (A). After application of folate the P-wave was reduced (B) and after washing the control response was restored (C). The N-wave was unaffected by folate throughout.

On the right, individual measurements of the P-wave are plotted as percentage of the control potential size against elapsed time during the experiment. Letters (A, B, C) above the graph signify measurements corresponding to the individual potentials shown on the left.

second application of folic acid solution produced a similar depression and a subsequent application of picrotoxin (0.2% w/v) also produced a similar degree of P-wave attenuation.

Discussion

The results reveal that folic acid will consistently antagonize presynaptic inhibition in the rat cuneate nucleus. It has been suggested that this inhibition is mediated by γ-aminobutyric acid (GABA) released from nerve terminals of interneurones in the nucleus (Davidson & Southwick, 1971) and that the antagonism of this inhibition by picrotoxin and bicuculline is a consequence of blockade of these GABA receptors. The possibility, therefore, arises that folic acid may be operating by blocking GABA receptors in a similar manner.

Microiontophoresis experiments in the rat cortex and cuneate nucleus (Hill *et al.*, 1974) have shown no evidence of GABA antagonism by folic acid, although work in the cat cortex (Davies & Watkins, 1973) indicated that GABA antagonism may have occurred on some neurones. Recent experiments suggest that the receptors mediating presynaptic inhibition in the cuneate are rather easier to block with picrotoxin and bicuculline than those with which microiontophoretically applied GABA combines (Hill, Simmonds & Straughan, 1973). The results of the present experiments would indicate that the same is true for folic acid.

Whether or not the folic acid is combining directly with presynaptic GABA receptors remains to be determined, but it is clear that the observed reduction of presynaptic inhibition could contribute substantially to the convulsant properties of this interesting substance and our findings support the previous report of a similarity between the convulsant actions of folic acid and picrotoxin in mice (Baxter, Miller & Webster, 1973).

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