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The influence of anticonvulsant drugs on formyl tetrahydrofolic acid stimulation of rat brain respiration *in vitro*

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THE TREATMENT of anti-epileptic drug-induced megaloblastic anaemia with folic acid may aggravate epilepsy[3]. A possible explanation of this is that folic acid, or one of its derivatives, may directly affect neuronal excitability. In favour of such a possibility, 5-fluorouracil (which blocks the utilization of active folate) lowers the convulsive threshold in rats.[1,4] Also formyl tetrahydrofolic acid (f-THF) in the presence of noradrenaline (NA) stimulates the respiration of brain synaptosomes and restores the respiration of synaptosomes which have been inhibited with phenobarbitone or phenytoin.[6] The following is an *in vitro* experiment in which the actions of phenobarbitone, phenytoin, primidone and sulthiame on brain respiration are observed and the influence of these drugs on f-THF stimulation of cerebral oxygen uptake examined.

Young adult, female, white Wistar rats weighing 150–190 g were used. A mitochondrial–synaptosomal suspension in 0·25 M sucrose was prepared and its oxygen consumption measured in an oxygen electrode as previously described.[6] The composition of the incubating medium was: glucose 10 mM; NaCl, 124 mM; KCl, 5 mM; KH_2PO_4 1·2 mM; $MgSO_4$, 1·3 mM; $CaCl_2$, 0·75 mM; NaH_2PO_4-Na_2H PO_4 buffer (pH 7·4) 5 mM. The final volume was 2·0 ml. To this solution various additions were made in volumes up to 0·2 ml. These gave final concentrations of up to the following values:

NA	7×10^{-4}M
f-THF	7×10^{-4}M
Primidone	$2{\cdot}5 \times 10^{-4}$M
Sulthiame	$2{\cdot}3 \times 10^{-4}$M
Phenytoin	$8{\cdot}0 \times 10^{-4}$M
Phenobarbitone	$8{\cdot}0 \times 10^{-5}$M

Concentration–response curves of enhancement of oxygen consumption by NA and f-THF are shown in Fig. 1. After the initial (ascending steeply) part of the curves, that for NA continued to rise but at a diminished rate, whereas that for f-THF reached a maximum response at about 4×10^{-4}M and above this concentration produced less effect.

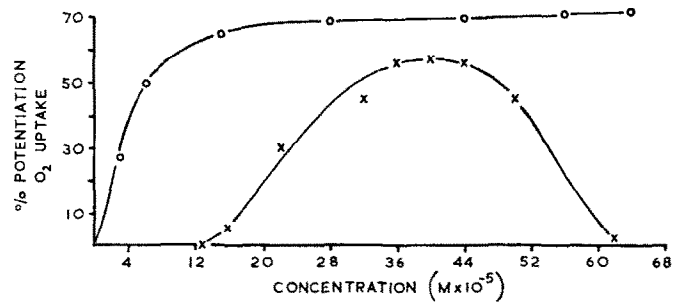

FIG. 1. Concentration of f-THF (×——×) and NA (○——○) plotted against enhancement of oxygen uptake of rat brain mitochondrial synaptosome preparations *in vitro*.

In the absence of added NA or f-THF, phenobarbitone, phenytoin, primidone, sulthiame and hyoscine inhibited oxygen uptake of the rat brain mitochondrial–synaptosomal preparation. Table 1 shows the concentration of these anticonvulsants necessary to produce 50 per cent inhibition of oxygen uptake and the minimum concentration necessary for complete inhibition of respiration. The cor-

TABLE 1. REVERSAL OF DRUG INDUCED INHIBITION OF *in vitro* RESPIRATION OF RAT BRAIN SYNAPTOSOMES BY f-THF.

Drug	Concn for 50% inhibition	Concn for complete inhibition	Concn of f-THF to reverse complete inhibition
Phenobarbitone	$3{\cdot}3 \times 10^{-5}$ M	$6{\cdot}0 \times 10^{-5}$ M	$1{\cdot}6 \times 10^{-4}$ M
Phenytoin	$3{\cdot}4 \times 10^{-5}$	$4{\cdot}9 \times 10^{-5}$	$9{\cdot}5 \times 10^{-5}$
Primidone	$5{\cdot}7 \times 10^{-5}$	$1{\cdot}5 \times 10^{-4}$	$2{\cdot}5 \times 10^{-4}$
Sulthiame	$3{\cdot}4 \times 10^{-5}$	$1{\cdot}0 \times 10^{-4}$	$3{\cdot}8 \times 10^{-4}$

Each value mean of 10 experiments.

responding values for hyoscine were $7{\cdot}4 \times 10^{-5}$ and $1{\cdot}6 \times 10^{-4}$M respectively. The addition of f-THF or NA or both did not affect the inhibition due to hyoscine (Fig. 2) but either of these reagents could completely reverse complete block in respiration due to the anti-convulsant agents (Table 1; Fig. 3). With these four anti-epileptic drugs, the concentration of f-THF necessary for complete

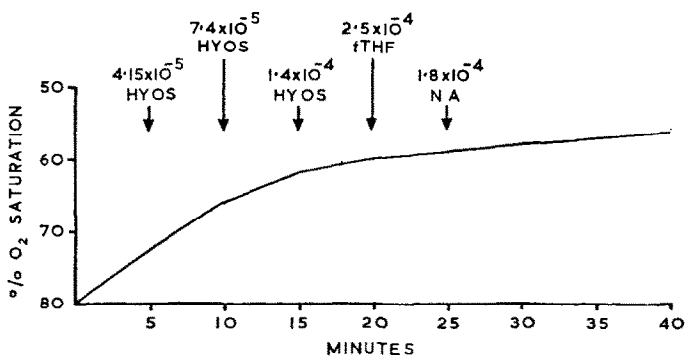

FIG. 2. Oxygen electrode tracing of oxygen consumption by a mitochondrial–synaptosome preparation showing the influence of hyoscine (HYOS), f-THF and NA on this. (Concentration of drugs expressed as molarity.)

reversal of inhibition of respiration was approximately twice that of the anti-convulsant drug. The concentration–response curve of f-THF, in the absence of other drugs showed no effect at concentrations below $1{\cdot}3 \times 10^{-5}$M and a maximum effect at $4{\cdot}0 \times 10^{-4}$M. This relationship was modified in the presence of an anti-epileptic drug. Figure 3, for example, shows complete respiratory block by $1{\cdot}5 \times 10^{-4}$M primidone. No observable effect resulted from the addition of $6{\cdot}34 \times 10^{-5}$M f-THF but complete removal of inhibition occurred with $2{\cdot}54 \times 10^{-4}$M f-THF. Thus the presence of the anti-epileptic drug raised the threshold for f-THF stimulation. On raising the concentration of primidone to a final molarity of $2{\cdot}4 \times 10^{-4}$ the partial respiratory inhibition which resulted was not reversed by increasing the concentration of f-THF to $4{\cdot}13 \times 10^{-4}$ and $5{\cdot}39 \times 10^{-4}$ M. In any of these experiments increasing the concentration of f-THF beyond $4{\cdot}0 \times 10^{-4}$ M resulted in no further enhancement of oxygen consumption rate in the presence or absence of an anticonvulsant drug. However even in the presence of supramaximal concentrations of f-THF, NA can still produce further stimulation of respiration (Fig. 3). The concentration–inhibition curves for the four anti-convulsants were sigmoidal, with a threshold value below which no respiratory inhibition occurred. For all the drugs this threshold lay between $1{\cdot}0$ and $3{\cdot}0 \times 10^{-5}$ M.

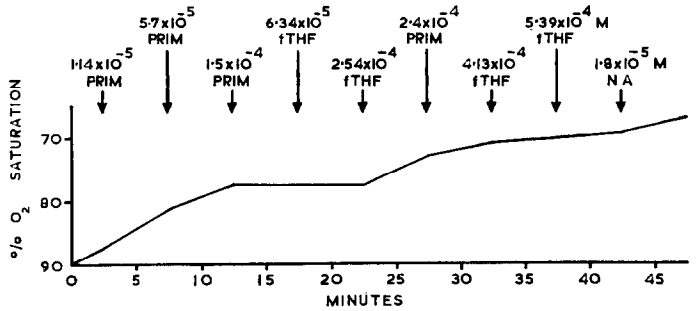

FIG. 3. As in Fig. 2, showing the influence of primidone (PRIM), f-THF and NA on oxygen consumption.

This experiment showed that the anti-epileptic drugs phenobarbitone, phenytoin, primidone and sulthiame all inhibited the respiration of a rat brain synaptosome-mitochondrial preparation at concentrations of between 3×10^{-5} and $1{\cdot}5 \times 10^{-4}$ M. These concentrations are similar to those found in body fluids following administration of the drug in man.[2] The drug primidone which is present in the highest concentration in the plasma in clinical situations (up to 2×10^{-4} M has been recorded[2]) required the highest concentration *in vitro* in the present experiments ($1{\cdot}5 \times 10^{-4}$ M) for complete arrest of respiration.

The inhibition of oxygen uptake by each of the anticonvulsants was reversed by the addition of f-THF. Such an effect supports the possibility that these drugs and f-THF act competitively in the brain. Thus, f-THF could be a stimulant of neuronal activity and the anti-epileptic drugs could act by antagonizing this action. Previous work has shown that 10^{-4} M chlorpromazine and 4×10^{-5} M prochlorperazine inhibit synaptosomal respiration *in vitro* and that this is not reversed by f-THF.[6] In the present experiment hyoscine, another sedative which is not specifically anti-epileptic, also produced inhibition of respiration at concentrations of approximately 7×10^{-5} M which could not be reversed with f-THF.

The concentrations of f-THF which will antagonize these actions of the anti-epileptics is approximately twice that of the latter (Table 1). However, the maximum effective concentration of f-THF in this situation is 4×10^{-4} M. One consequence of this is that above concentrations of approx. 2×10^{-4} M of anti-epileptics, complete reversal by f-THF cannot occur. It is of interest that 4×10^{-4} M is also the maximum stimulating concentration of f-THF in the absence of anti-epileptic drugs. This in itself would suggest that the f-THF and anti-epileptic drugs act at independent sites in the brain preparation. The ability of f-THF to completely reverse total inhibition of respiration by these drugs would however indicate a competitive action on a common site, if in fact the kinetics of the situation were of the Michaelis–Menten type. This is not the case, the concentration–response curves for both f-THF and all the anti-epileptic drugs tested are sigmoidal in shape. Also, other experiments have demonstrated that the Hill plot for the stimulatory action of f-THF on rat brain respiration *in vitro* has a gradient greater than unity.[6] Similarly, further increases in the rate of oxygen consumption can be produced by NA even when the maximum effect of f-THF has been reached. This indicates

separate receptor sites for NA and f-THF. However, the fact that there is considerable mutual potentiation of these two substances[5] suggests interaction between the two sites of action.

Although these results clearly indicate an antagonism between anti-epileptic drugs and f-THF and NA on the respiratory rate of a brain preparation, the relevance of this to the *in vivo* situation is not known.

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Effects of potential gradient on the electrophoretic mobility of human blood platelets in the presence of ADP or noradrenaline

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IN THROMBOGENESIS platelets stick to the wall of a blood vessel where they attract other platelets, and because they normally carry a net negative charge and are mutually repellent it is thought that in thrombogenesis or platelet aggregation *in vitro* the platelet surface charge is somehow reduced. Such a change should be reflected in the mobility of the platelets in an electric field, and electrophoresis has been used to measure it.

The effects of various aggregating agents on the electrophoretic mobility of platelets from healthy people and patients with various diseases have been studied by several groups of workers, but the results are conflicting. According to Hampton and Mitchell[1] low concentrations of adenosine diphosphate (ADP) or noradrenaline increase the mobility of normal platelets while higher concentrations reduce it. These workers[2,3] claimed that the concentrations of ADP and noradrenaline required to produce the maximum increase in mobility are decreased in various acute illnesses, whereas in arterial diseases the sensitivity to ADP is increased but that to noradrenaline is normal. Gröttom,[4] however, was unable to confirm the claim that low concentrations of either aggregating agent increase mobility: he maintained that the two compounds can only decrease mobility. Furthermore he was unable to demonstrate any differences between healthy people and patients with vascular disease in the way their platelet mobilities responded to the two aggregating agents. Rutty and Vine,[5] attempting to resolve these contradictory claims, obtained results on normal platelets which they said supported those of Hampton and Mitchell.[1] All three sets of results on normal platelets are given in Table 1.

Electrophoretic studies on platelets offer much promise in the diagnosis of disease and in the elucidation of mechanisms by which platelet behaviour is changed, but the present situation of contradictory claims is disappointing. As Turpie, McNicol and Douglas[6] have opined in their review of this field "further work is required to resolve this issue".

Hampton and Mitchell[1–3] used platinum electrodes and a capillary tube micro-electrophoresis apparatus developed by Bangham *et al.*[7] and manufactured by Rank Bros., Cambridge, England, whereas Gröttom[4] used copper–copper sulphate or silver–silver chloride electrodes and a flat rectangular cell apparatus developed by Ruhenstroth-Bauer[8] and made by the Zeiss Co., Oberkochen, Germany. Rutty and Vine[5] used the Rank model and silver–silver chloride electrodes. All three groups used citrated platelet-rich plasma (PRP) diluted with 9 vol. of citrated platelet-poor plasma (PPP). Although differences in apparatus and methodology have been emphasized and discussed by several workers[2,3,6] no explanation has emerged to reconcile these conflicting claims.

Having only a capillary tube microelectrophoresis apparatus I was unable to repeat Gröttom's