

Effects of formyl tetrahydrofolic acid and noradrenaline on the oxygen consumption of rat brain synaptosome-mitochondrial preparations

R. G. SPECTOR

Department of Pharmacology, Guy's Hospital Medical School, London

Summary

1. The respiration of a mitochondrial and synaptosome fraction of rat cerebral cortex was measured using an oxygen electrode.
2. Little or no enhancement of oxygen uptake occurred on separately adding formyl tetrahydrofolate or noradrenaline.
3. When these substances were added together a marked increase in respiration was observed.
4. Both phenobarbitone and phenytoin inhibited the respiration of this preparation, but this was reversed on the addition of formyl tetrahydrofolate.
5. The possibility that some antiepileptic drugs may compete with formyl tetrahydrofolate in the brain is considered and structural similarities between folic acid and these drugs are indicated.

Introduction

Many antiepileptic drugs are capable of producing megaloblastic anaemias in man (Badenoch, 1954). This anaemia responds to folic acid (Hawkins & Meynell, 1954), but such treatment may aggravate the epilepsy (Dennis & Taylor, 1969). Therefore it is possible that pteroyl glutamate (folic acid) may play a role in cerebral excitability and that the antiepileptic drugs act as antagonists to both the central action and the peripheral utilization of pteroyl glutamate. The following is an investigation of the influence of pteroyl glutamate and its formyl tetrahydro metabolite on brain respiration *in vitro*, which was undertaken to examine the possibility that they can stimulate neuronal activity.

Methods

Young adult white Wistar rats (140-200 g) of either sex were used. The cerebral cortex (0.4-0.5 g) was homogenized in a glass and Teflon homogenizer in ice cold 0.25 M sucrose (1 g/10 ml). The suspension was centrifuged at 900 g for 10 min and the resulting supernatant was centrifuged at 10,000 g for 20 minutes. The pellet (150 ± 50 mg) was resuspended and washed twice in sucrose. The final pellet was suspended in sucrose (25 mg/ml). Electron microscopy showed this preparation to contain mainly free mitochondria and synaptosomes. Oxygen consumption of the preparation was measured using a Rank oxygen electrode and a Smith's Servoscribe recorder (Glasstone & Lewis, 1964). For this, 0.1 ml of the sucrose suspension diluted to 2.0 ml with medium, were placed in the well of the electrode which was main-

tained at 37° C by circulating water from a water bath around it. The contents of the well were continuously mixed with a 5 mm magnetic stirrer and oxygenated with air for 1 minute. After oxygenation the mouth of the well was occluded by a Perspex plunger.

The composition of the incubation 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_2HPO_4 buffer (pH 7.4), 5 mM. The final volume was 2.0 ml.

In the experiments additional substances were added to the well in volumes of 1–100 μl , giving the following final concentrations: formyl tetrahydrofolic acid calcium salt, 0.0016–0.635 mM; folic acid (pteroyl monoglutamic acid sodium salt; pteroyl glutamate) 0.0016–0.635 mM; DL-noradrenaline tartrate (NA), 0.0015–0.3 mM; DL-isoprenaline sulphate, 0.0015–0.3 mM; DL-adrenaline, 0.0015–0.3 mM; dopamine hydrochloride, 0.015–0.3 mM; acetylcholine chloride, 0.0001–0.1 mM; histamine diphosphate, 0.0015–0.3 mM; phenobarbitone sodium, 0.0001–1.5 mM; phenytoin sodium, 0.0001–1.5 mM; chlorpromazine hydrochloride 0.0001–1.5 mM; prochlorperazine methanesulphonate, 0.00005–0.5 mM.

Results

The separate addition of either formyl tetrahydrofolic acid (f-THF) or noradrenaline (NA) either did not affect the respiratory rate of the brain (synaptosome-mitochondrial) preparation or produced only a small degree of enhancement. On the other hand, the presence of both f-THF and noradrenaline resulted in a sustained stimulation of oxygen uptake (Fig. 1). However, although the brain fraction utilized oxygen at a constant rate for at least 6 h following completion of its preparation (stored at 4° C), the ability to be stimulated by a combination of f-THF and noradrenaline persisted for only 2–3 h after completion of the preparation (stored at 4° C).

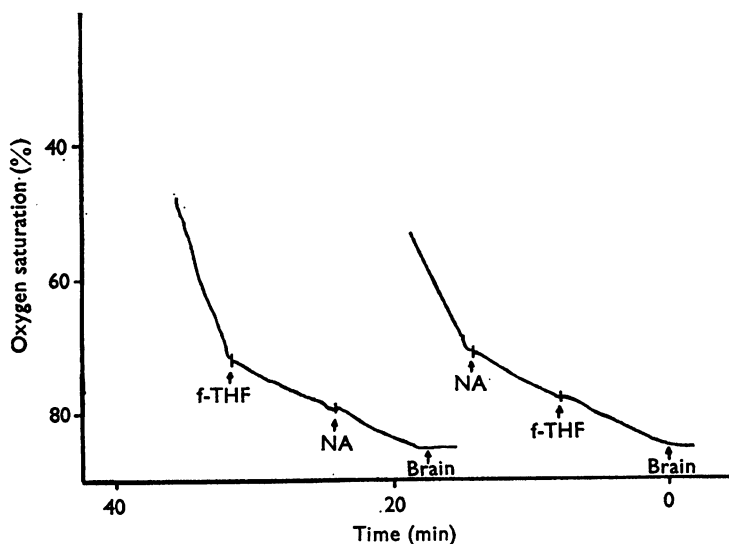


FIG. 1. Oxygen consumption of rat brain synaptosome-mitochondrial fraction measured in an oxygen electrode calibrated with aqueous solutions of known oxygen saturation. The figure shows the effects of f-THF ($6.3 \times 10^{-5}\text{M}$) and noradrenaline ($6 \times 10^{-5}\text{M}$).

Adrenaline, but not isoprenaline, also enhanced the rate of oxygen uptake in the presence of f-THF (Fig. 2). No effect on respiration in the presence of $0.0016\text{--}0.635$ mM f-THF was observed on the addition of pteroyl glutamate ($0.0016\text{--}0.635$ mM), acetylcholine ($0.0001\text{--}0.1$ mM), histamine ($0.0015\text{--}0.3$ mM) or dopamine ($0.0015\text{--}0.3$ mM). The same applied for consecutive additions of pteroyl glutamate ($0.0016\text{--}0.635$ mM) and noradrenaline ($0.0015\text{--}0.3$ mM).

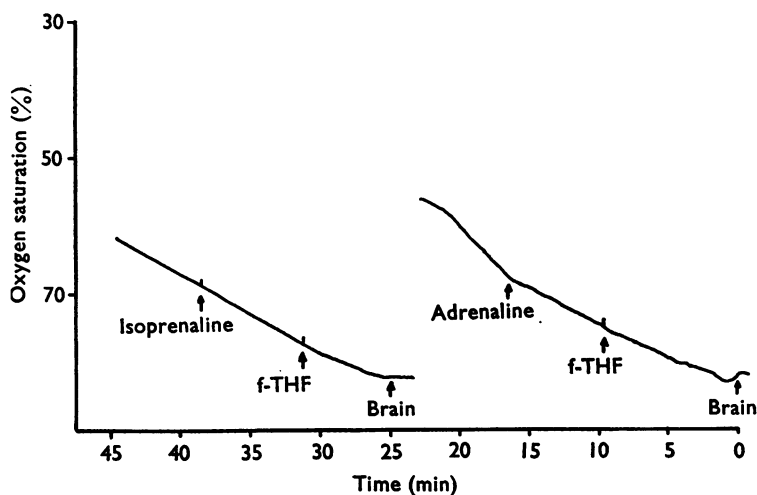


FIG. 2. Oxygen consumption of rat brain synaptosome-mitochondrial fraction in the presence of f-THF (6.3×10^{-5} M) with adrenaline (6×10^{-5} M), or isoprenaline (6×10^{-5} M).

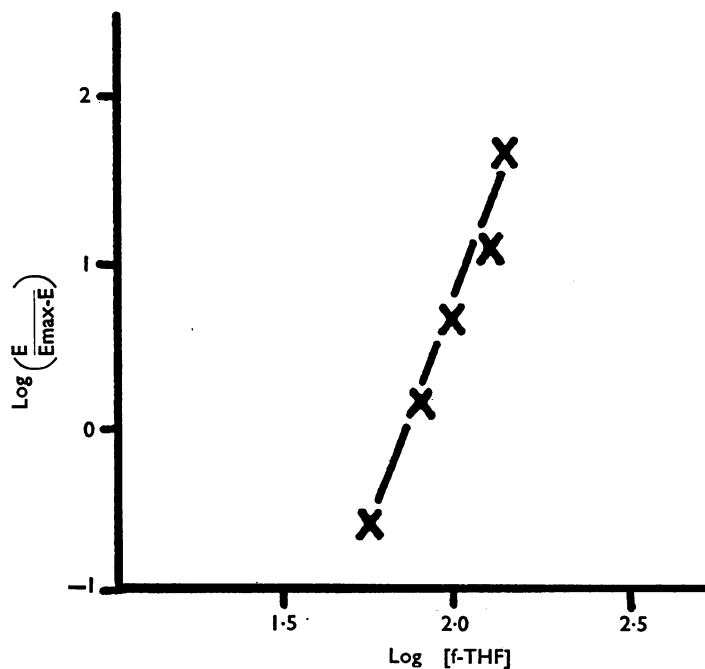


FIG. 3. Hill plot of results obtained when the concentration of f-THF was varied and the concentration of noradrenaline was kept constant (0.06 mM). E_{max} is the maximum effect obtainable and E the effect observed. $n=5.3$.

Concentration-response curves for experiments with noradrenaline and f-THF were sigmoidal when the concentration of one of them was varied and that of the other remained constant. Satisfactory reproducible data could only be obtained if the observations were made within 1 h of completing the preparation. On varying the concentration of f-THF from 0.0016–0.635 mM and maintaining the concentration of noradrenaline constant (0.06 mM), Hill plots were obtained with a mean gradient of 5.9 ± 0.4 (S.E.M.) (Fig. 3). Linear Hill plots were also obtained when the f-THF concentration was maintained at 0.064 mM and the noradrenaline concentration varied between 0.015 mM and 0.3 mM. The mean gradient was 2.6 ± 0.5 (S.E.M.).

In the presence of 0.06 mM noradrenaline, 0.3 mM phenobarbitone or 0.3 mM phenytoin produced 20–50% inhibition of the rate of oxygen uptake. This was immediately restored to control values by 0.2 mM f-THF. Chlorpromazine (0.1 mM), and prochlorperazine (0.04 mM), produced 50–80% inhibition of respiration in the presence of 0.06 mM noradrenaline but this effect was not reversed by the addition of up to 0.62 mM f-THF.

Discussion

These experiments indicate that under certain conditions f-THF stimulates the respiration of synaptosome-mitochondrial preparations of the rat brain. Clearly this does not necessarily mean that f-THF stimulates cerebral electrical activity. However, one possible cause of enhanced respiration could be increased neuronal activity.

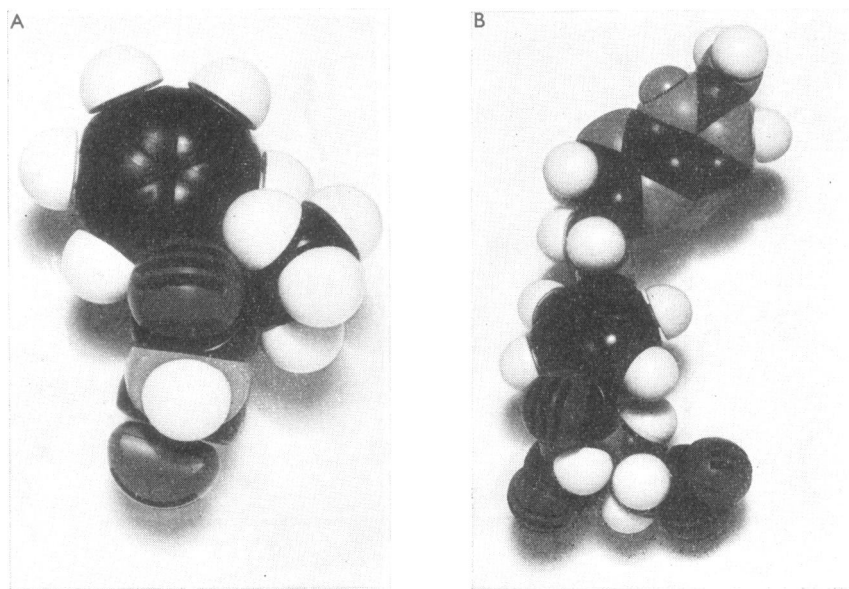
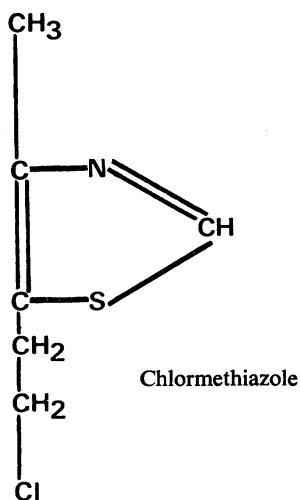


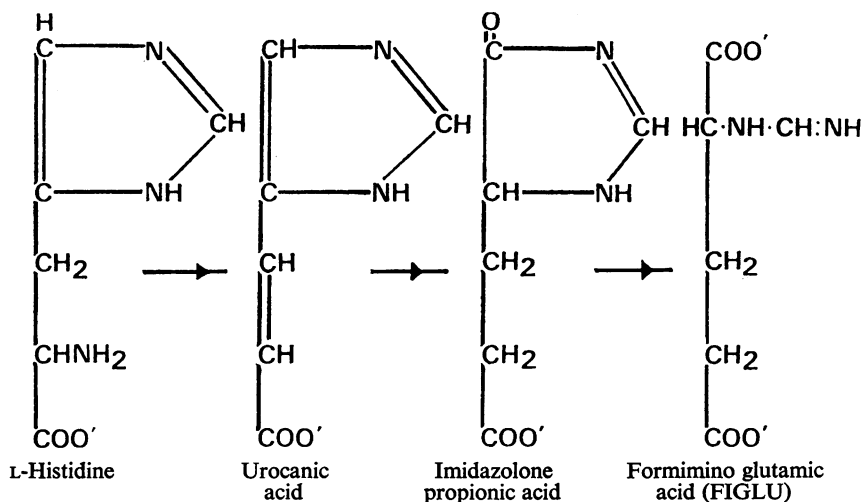
FIG. 4. Improved Corey-Pauling space filling models of the molecular structures of phenobarbitone (A) and pteroyl glutamate (B).

Assuming f-THF is an excitatory metabolite in the brain, and that for this action noradrenaline must be present, the antiepileptic drugs could produce their central effect by competing with f-THF. This work indicates a competition between antiepileptic drugs and f-THF induced stimulation of brain respiration. Suggestive evidence for competition is provided by an examination of the structures of several antiepileptic drugs. Phenobarbitone (Fig. 4a) contains an aromatic 6-membered ring adjacent to which is a carbon atom bearing a carboxyl oxygen (belonging to the ureide ring). Also in relation to the carboxyl group are a protonated nitrogen atom and a compact hydrocarbon group (ethyl). These features are also present in diazepam and phenytoin, both of which have as the hydrocarbon group a benzene ring, and in phenacemide and primidone in which the hydrocarbon moieties are methyl and ethyl groups, respectively. The tricyclic antiepileptic drug nitrazepam also shows these features, whereas the structurally similar drug chlorpromazine, which is not antiepileptic, does not contain a carboxyl group with an adjacent nitrogen. Sulthiame is of particular interest as it has no carboxyl group, but a double bonded oxygen which is held in the appropriate site by a sulphur atom. All these features are present in the pteroyl glutamate molecule. The aromatic 6-membered ring is that of the *p*-amino benzoic acid portion, the carboxyl and amine groups are present in the peptide bond linking *p*-amino benzoic acid and glutamic acid and the hydrocarbon portion is represented by the remaining 5 carbons of the glutamate. The general arrangement of these features in the pteroyl glutamate molecule is similar to that seen in the antiepileptic drugs (Fig. 4b). It is perhaps significant that increasing the hydrocarbon side chain substituent in barbiturates beyond 5–6 carbons results in a loss of central nervous depressant activity and the appearance of epileptogenic properties. If the glutamate portion of pteroyl glutamate and the compact hydrocarbon group of the antiepileptic drugs do in fact compete for the same area of a receptor macromolecule, then the latter group might only fit if it contained 6 carbon atoms or less.

The anticonvulsant drug chlormethiazole does not show any of the above structural features:



However, it does exhibit structural analogies with imidazolone propionic acid, which is involved in the production of the formate group of f-THF:



FIGLU + tetrahydrofolate → 5 formimino tetrahydrofolate + glutamate.

5 Formimino tetrahydrofolate → 5,10 methenyl tetrahydrofolate + NH₃.

5,10 Methenyl tetrahydrofolate + H₂O → 10 formyl tetrahydrofolate + H⁺.

If the antiepileptic properties of chlormethiazole are due to a competitive inhibition of the utilization of imidazolone propionic acid and a resultant decrease in the rate of synthesis of f-THF, it is probable that this drug could cause a megaloblastic anaemia. This has apparently not been observed in patients treated with the drug, but there are no records of individuals taking chlormethiazole for long periods.

Since a high proportion of drugs which are effective in the treatment of grand mal epilepsy can produce a pteroyl glutamate (folic acid) deficiency anaemia, it is important to consider also that these drugs might exert their central effect by an inhibition of cerebral pteroyl glutamate metabolism or that pteroyl glutamate may itself directly influence brain excitability. Drugs which block the conversion of pteroyl glutamate to f-THF alter the threshold to convulsions in the rat. For instance, methotrexate protects against leptazol fits (Spector, 1971). On the other hand 5 fluorouracil, which blocks the utilization of f-THF in the thymidylate synthetase reaction, lowers the convulsive threshold (Carlisle & Spector, 1971; Spector, 1971). This suggests that the rates of production and metabolism of f-THF are both important. In the present work neither pteroyl glutamate nor f-THF affected the respiration of the brain preparation but in the presence of noradrenaline, f-THF but not pteroyl glutamate produced a marked stimulation of oxygen uptake. These findings do not rule out the possibility that *in vivo* antiepileptic drugs affect the concentration of f-THF. Palmer, Robison & Sulser (1971) found that some centrally acting drugs profoundly affected the ability of noradrenaline to raise the concentrations of cerebral cyclic adenosine monophosphate (AMP), whereas little effect on cyclic AMP was observed in the absence of noradrenaline. The importance of cyclic AMP in brain function is not known; however, Ginsborg & Hirst (1971) consider that this substance is necessary for transmitter release and they have provided

indirect evidence that postjunctional membrane electrical activity is increased by cyclic AMP. If this occurs *in vitro* the consumption of adenosine triphosphate and appearance of AMP would result in an enhancement of oxygen consumption.

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