

SHORT COMMUNICATIONS

Folate, catecholamines and bacterial respiration

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Folate has a stimulatory effect on several types of mammalian tissue: neuromuscular transmission, cardiac contractility and CNS excitability are all enhanced. The mechanisms by which these effects are produced are not understood, but the end results are in general similar to those produced by catecholamines [1,2].

In order to investigate other cell types and study homogenous cell populations, the influence of these agents on bacteria was observed.

Organisms from a single colony were incubated overnight on fresh blood agar, thrice washed and suspended in isotonic saline. Oxygen uptake of the organisms was measured in Rank oxygen electrode cell using an incubation mixture consisting of: glucose 10 mM; NaCl 124 mM; KCl 5 mM; KH_2PO_4 1.2 mM; Mg 50 g 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.

The respiration of *Klebsiella aerogenes*, *Bacillus proteus*, *Pseudomonas pyocyanea* and *Escherichia coli* was greatly stimulated by approx $0.7\text{--}10 \times 10^{-4}$ M formyl tetrahydrofolic acid (f-THF) but not by $0.5\text{--}10 \times 10^{-4}$ M folic acid (pteroyl monoglutamate; PGA). Stimulation by f-THF was blocked by the anti-convulsant drugs phenytoin, sulthiame and carbamazepine ($0.1\text{--}1 \times 10^{-4}$ M). In the absence of f-THF, up to 10^{-4} M concentrations of these drugs did not affect respiration. Figure 1 shows the stimulation of oxygen uptake of *Klebsiella pneumoniae* by f-THF. As in all these experiments, the respiratory response to increasing concentrations is sigmoid, with little or no stimulation below 10^{-4} M f-THF. Above $0.4\text{--}1 \times 10^{-3}$ M f-THF, inhibition of oxygen consumption occurs.

Of the Gram positive organisms, *Streptococcus faecalis* and *Staphylococcus pyogenes* showed no significant respiratory change with f-THF. *Bacillus subtilis* however, showed a twofold stimulation of respiration with 5.5×10^{-4} M f-THF, which was reversed by $0.5\text{--}1 \times 10^{-4}$ M phenytoin or carbamazepine. Adrenaline and noradrenaline ($3\text{--}20 \times 10^{-5}$ M) but not isoprenaline produced up to a 6-fold increase in oxygen uptake by all the organisms tested. These two catecholamines also restore oxygen consumption in bacteria whose respiration has been inhibited by phenytoin. However, folate and catecholamines appear to enhance respiration by different mechanisms, because bacteria exposed to supramaximal concentrations of f-THF (2×10^{-3} M) can still be further stimulated by adrenaline and noradrenaline. 3',5'-cyclic AMP (cAMP) and dibutyryl cAMP do not produce stimulation of respiration when added in concentrations up to 10^{-3} M.

Other central depressants which are not anticonvulsants were also tested in this system. Chlorpromazine and pro-

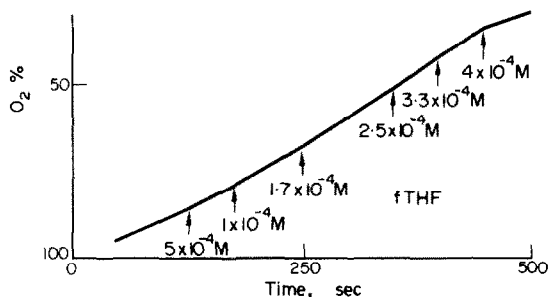


Fig. 1. The effect of increasing concentrations of folinic acid (f-THF) on oxygen consumption of *Klebsiella aerogenes*.

methazine ($1 \times 10^{-5}\text{--}5 \times 10^{-4}$ M) produced a stimulation followed by inhibition of oxygen uptake on all organisms tested. This was not reversed by f-THF.

These experiments indicate that f-THF stimulates bacterial respiration. The response is immediate (Fig. 1) and is therefore not likely to be due to stimulation of cell division by providing 1-carbon carriers for nucleic acid synthesis. Such an immediate response is reminiscent of the stimulatory action of folate on heart [3] and other mammalian excitable tissues. Another similarity between the mammalian and bacterial response is that the effects of f-THF and anticonvulsant drugs are mutually competitive, also non anti-convulsant cerebral depressants do not behave in this way. Although the bacterial respiration responds in a similar manner to some catecholamines and f-THF, this experiment indicates that the mechanism for these effects is different.

These results support the concept that $10^{-3}\text{--}10^{-5}$ M f-THF has actions on many types of cell, possibly by affecting mechanisms other than the established role of folate in one-carbon transfer reactions.

Department of Pharmacology,
Guy's Hospital Medical School,
London SE1 9RT,
England

D. JENKINS
R. G. SPECTOR

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