The anti-sickling activity of a series of aromatic aldehydes

C.R. BEDDELL, G. KNEEN & R.D. WHITE

Wellcome Research Laboratories, Beckenham Kent BR3 3BS, U.K.

Human sickle cell anaemia is primarily caused by the relative insolubility of sickle cell deoxyhaemoglobin (deoxy-HbS) compared with that of normal deoxy-HbA. If the intraerythrocytic concentration of deoxy-HbS reaches a critical level during a cycle of deoxygenation, the protein can aggregate into polymers which distort the erythrocyte to give the characteristic sickle cell appearance.

A rational approach to therapy is to alter the relative proportions of oxy- and deoxy-HbS in venous blood, not so extensively as to compromise tissue oxygenation but sufficiently to prevent the concentration of deoxy-HbS from reaching the critical level for sickling. Aromatic aldehydes are known to form Schiff base adducts with free amino groups of haemoglobin; this mechanism can stabilise oxyhaemoglobin and shift the oxygen dissociation curve to the left. Zaugg, Walder & Klotz (1977) have demonstrated this phenomenon using normal erythrocytes and showed that two of the most potent 'left-shifting' aldehydes, o-vanillin and salicylaldehyde, also reduced the incidence of sickling in whole sickle blood.

We have also examined a small series of aromatic aldehydes to see whether anti-sickling activity in whole sickle blood can generally be correlated with left-shifting potency in normal blood.

Sickle blood (1 ml) was mixed with bicarbonate-buffered saline (1 ml) containing sufficient compound to give a total concentration of 3 mM. Incubation was conducted at 37°C, pH 7.3, under an atmosphere of humidified air/5% CO_2. After 60 min the gaseous phase was changed to humidified 4% O_2/91% N_2/5% CO_2 and incubation was continued until a new equilibrium oxygen tension (approx. 25 mm Hg) was established. Samples of blood were fixed in 3% formal phosphate-buffered saline and photomicrographs were prepared. Fields were counted by two independent observers, who classified a minimum of 600 cells/sample as either Normal, Sickle or Bizarre. Sickling of 30–50% was observed in controls, the actual figure being donor and experiment dependent.

Compounds were ranked in two ways: (i) ability to reduce sickling and (ii) ability to produce an increase in the proportion of normal cells. Rank orders of potency were subjected to non-parametric analysis of variance (Hutchinson, 1977). The final order, for anti-sickling activity, was

o-vanillin $\simeq$ 2,3 dihydroxybenzaldehyde $\simeq$ salicylaldehyde > 2,4 dihydroxybenzaldehyde > p-vanillin > m-anisaldehyde $\simeq$ p-hydroxybenzaldehyde > guaiacol. o-Vanillin abolished sickling, whereas guaiacol was ineffective at 3 mM (the latter is not an aldehyde).

The results agree closely with the findings of Zaugg *et al.* (1977) and confirm that measurements of left-shift can reveal potential anti-sickling agents. However, when the compounds were ranked by proportion of normal cells, o-vanillin was appreciably better than salicylaldehyde which demonstrates that it is important to consider not only sickle but bizarre forms as well.

We thank Drs. P.J. Goodford, O.M. Gyde, G.P. Taylor and Prof. J.M. White for advice and help, and Miss J. Ingram and Mr K. Patel for valued technical assistance.

References

HUTCHINSON, T.P. (1977). The method of m rankings where the total number of observations in each cell are not unity. *Comput. & Biomed. Res.*, **10**, 345–361.

ZAUGG, R.M., WALDER, J.A. & KLOTZ, I.M. (1977). Schiff base adducts of haemoglobin. *J. biol. Chem.*, **252**, 8542–8548.

Lithium effects on purified rat brain pyruvate kinase

N.J. BIRCH, R.P. HULLIN, P. KAJDA & M.J. O'BRIEN

Department of Biochemistry, University of Leeds

We have shown inhibition by lithium of the activity of magnesium dependent enzymes, for instance using commercially available rabbit muscle pyruvate kinase (Birch, Hullin, Inie & Leaf, 1974; O'Brien, Allin, Birch & Hullin, 1977). The relevance of such findings to the biochemical pharmacology of lithium in manic depressive psychoses is not clear and studies on pyruvate kinase from brain might provide additional information.

Pyruvate kinase was prepared from 35 g of pooled brains from untreated Wistar rats using a method based on that of Parkinson & Easterby (1977). After homogenisation at 4°C pyruvate kinase was obtained in the supernatant of a phosphate buffer extract and purified by chromatography at pH 6.5 on a phosphocellulose column. This was followed by concentration in an ultrafiltration cell and the enzyme fraction was chromatographed on a column of DEAE cellulose at pH 8.0 using a linear potassium chloride gradient. Final purification was obtained using Sephadex G200 at pH 8.0 when the yield was 11.5% of the original supernatant enzyme.

The molecular weight of pyruvate kinase obtained by this method was found to be 210,000 ± 20,000 Daltons by sedimentation equilibrium in an ultracentrifuge. Studies of the molecular weights of the subunits suggest that the purified enzyme is of the same type (M) as that of the muscle enzyme used in previous studies, (O'Brien *et al.*, 1977).

The effect of lithium on this purified preparation was similar to that previously reported (Birch, 1978). Lithium inhibition was noncompetitive with respect to Mg^{2+}, K^+ and phosphoenol pyruvate but was competitive with ADP. The degree of inhibition by lithium of the purified brain enzyme was higher than for the muscle enzyme. Brain pyruvate kinase was inhibited by 7–12% by lithium (2 mmol/l) and ADP (1 mmol/l). With a higher concentration of lithium (10 mmol/l) in the presence of ADP (1 mmol/l), the degree of inhibition of pyruvate kinase was 25–32%. This is not in accord with the work of Balan, Cernătescu, Trandafirescu & Ababei (1974) who showed 75–94% inhibition of a crude extract of rat brain pyruvate kinase with 10 mmol/l lithium. These experiments were carried out under apparently identical conditions and we are unable to explain the discrepancy.

We conclude therefore that the inhibition by lithium of pyruvate kinase has been confirmed in an extract from brain and that such inhibition occurs at concentrations of lithium which might obtain during prophylactic lithium treatment.

References

BALAN, G., CERNĂTESCU, D., TRANDAFIRESCU, M. & ABABEI, L. (1974). Influence of lithium on hexokinase and pyruvate kinase activity. *Rev. Med-Chir. Soc. Med. Nat. Iasi*, **78**, 901–6.

BIRCH, N.J. (1978). Metabolic effects of lithium. In *Lithium in Medical Practice* Ed. Johnson, F.N. & Johnson, S. Pp. 89–114, Lancaster, M.T.P.

BIRCH, N.J., HULLIN, R.P., INIE, R.A. & LEAF, F.C. (1974). Effect of lithium on the activity of pyruvate kinase and other magnesium dependent enzymes. *Br. J. Pharmac.*, **52**, 139P.

O'BRIEN, M.J., ALLIN, C.N., BIRCH, N.J. & HULLIN, R.P. (1977). Inhibition of rabbit muscle pyruvate kinase by lithium. *Br. J. Pharmac.*, **61**, 121P–122P.

PARKINSON, J. & EASTERBY, J.S. (1977). Purification and characterisation of bovine heart pyruvate kinase. *Biochim. Biophys. Acta.*, **481**, 471–480.

The effect of light on the enhancement of bacterial respiration by formyl tetrahydrofolic acid

R.G. SPECTOR

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

Formyl tetrahydrofolic acid (fTHF) stimultes the activity of several types of excitable cell (Spector, 1972; Jenkins & Spector, 1973) and also increases the rate of oxygen consumption of bacteria (Jenkins & Spector, 1975).

In the present work the oxygen consumption of E coli was measured using an oxygen electrode. When the cell was shielded from light, concentrations of fTHF up to 10^{-2} M had no influence on respiration. Exposure to light – particularly at the blue end of the visible spectrum – produced an immediate state of responsiveness to fTHF. Concentrations down to 10^{-5} M produced a dose-dependent stimulation of respiration. Light had no effect on the oxygen consumption of the organisms in a glucose-Ringer solution only.

High concentrations (10^{-3} M) of barbiturates, phenothiazines and anticonvulsants were required to depress bacterial respiration under dark conditions in the presence of folate. However illuminated bacteria enhanced by fTHF were sensitive to 10^{-6} M concentrations of the cerebral depressants which were examined.

The rate of many chemical reactions is increased on exposure to light, presumably due to electron promotion changes. These experiments appear to be examples of pharmacological actions being enhanced by such photolytic events.

References

SPECTOR, R.G. (1972). Influence of folic acid on excitable tissues. *Nature New Biol.*, **240**, 247–249.

JENKINS, D. & SPECTOR, R.G. (1973). The actions of folate and phenytoin on the rat heart *in vivo* and *in vitro*. *Biochem. Pharmac.*, **22**, 1813–1816.

JENKINS, D. & SPECTOR, R.G. (1976). Folate, catecholamines and bacterial respiration. *Biochem. Pharmac.*, **25**, 487.