The anti-sickling activity of a series of aromatic aldehydes

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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.

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Lithium effects on purified rat brain pyruvate kinase

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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.