The effect of electrolytes on the reactivation of phosphorylated cholinesterase

The reactivation by hydroxamic acids or oximes of cholinesterase (ChE) inhibited with organophosphates has been shown to have much in common with more normal enzymic reactions. The kinetics of reactivation are consistent with a mechanism similar to that of MICHAELIS AND MENTEN[1] for enzyme–substrate interaction, while the specificity properties of reactivators are related to those of substrates for ChE[2–4]. A further well-known property of ChE-catalysed hydrolysis is the marked effect of electrolytes which reduce the apparent strength of binding between the enzyme and substrate and so increase the optimum substrate concentration, but increase the rate of breakdown of the enzyme–substrate complex[5–7]. In the present communication electrolytes are shown to have equally marked effects on the reactivation process, but whether the rate is increased or decreased depends on the chemical structure of the reactivator.

Washed human erythrocytes were allowed to stand for 10 min at 25° in an equal volume of 0.9% NaCl containing $10^{-6}M$ tetraethyl pyrophosphate (TEPP). The cells were then washed with ice-cold saline to remove excess TEPP and were then haemolysed by freezing in an equal volume of saline. 10 ml of the haemolysed solution were mixed with 10 ml of a solution of the reactivator in barbitone buffer (0.01 M sodium diethylbarbiturate, 0.002 M KH_2PO_4) containing an appropriate concentration of the required electrolyte. The pH was rapidly adjusted to 7.8 and the mixture was stored at 25°. At suitable time intervals 1 ml samples were withdrawn and analysed for ChE by the electrometric method[8]. The rate constants were calculated as described elsewhere[9] from the slope of a graph of log $(E_\infty - E_t)$ against time, where E_t is the fraction of enzyme reactivated after time t.

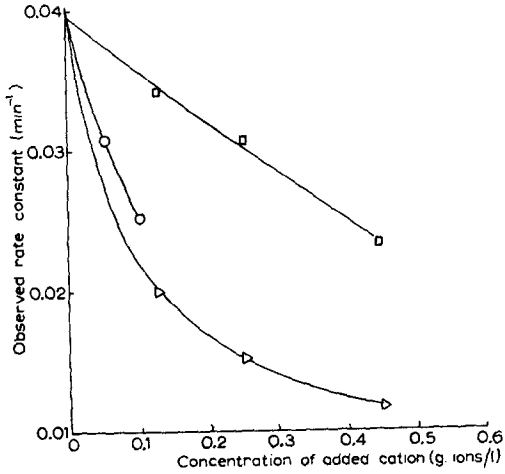

Fig. 1. The effect of cations on the reactivation by pyridine-2-aldoxime methiodide ($10^{-4}M$) at pH 7.8 and 25° of erythrocyte ChE inhibited with TEPP. □ KCl, △ NH_4Cl, ○ choline chloride.

Figs. 1 and 2 illustrate the effect of a range of electrolytes on reactivation by pyridine-2-aldoxime methiodide and by isonitrosoacetone. As can be seen, the effect of electrolytes is qualitatively different for the two compounds. Particularly noteworthy is the marked acceleration of reactivation by isonitrosoacetone due to potassium salts, the effects of the chloride and sulphate being dependent on the concentration of potassium ions but not on the concentration of the anion. This fact, together with the considerable variation in the magnitude of the effect due to different chlorides, suggests that the effect is predominantly due to the specific cation, as is the effect of electrolytes on the direct ChE-catalysed hydrolysis of acetylcholine[10]. MYERS[7] has attributed the shift in the optimum substrate concentration for the hydrolysis of acetylcholine by ChE in the presence of electrolytes to competition between the positively charged acetylcholine and the cation of the electrolyte for the anionic site on the enzyme surface. It has been shown[2,3] that reactivation by pyridine-2-aldoxime methiodide is greatly accelerated by the formation of a complex between the oxime and the inhibited enzyme, which are bound, at least partly, by attraction between the positively charged nitrogen atom in the oxime and the anionic site on the phosphorylated enzyme. It is thus reasonable to expect that cations generally would compete with pyridine-2-aldoxime methiodide for the anionic site and so retard reactivation. Fig. 1 shows that this retardation does occur.

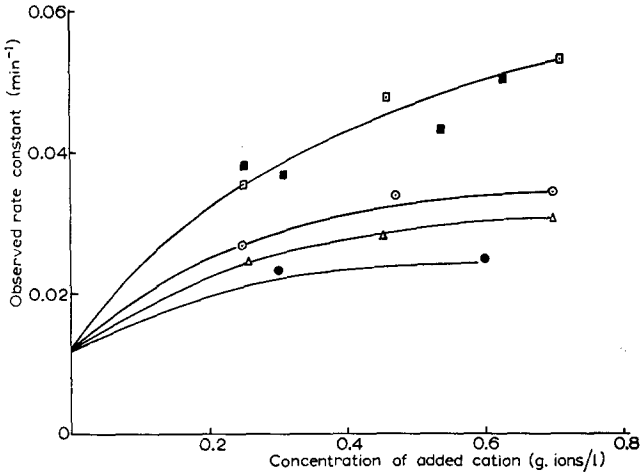

Fig. 2. The effect of cations on the reactivation by isonitrosoacetone (0.004 M) at pH 7.8 and 25° of erythrocyte ChE inhibited with TEPP. □ KCl, ■ K_2SO_4, ○ NaCl, ● Na_2SO_4, △ NH_4Cl.

Although reactivation by isonitrosoacetone is also accelerated by complex formation[2], the formation of this complex does not involve the anionic site on the enzyme, in which case the slowing down of reactivation due to cations, found with pyridine-2-aldoxime methiodide, would not necessarily be expected. In fact, Fig. 2 shows that cations, instead of retarding reactivation by isonitrosoacetone, markedly accelerate it. A similar acceleration due to cations is found when picolinhydroxamic acid is used as a reactivator. This difference between positively charged and uncharged reactivators is consistent with the belief[2] that they form complexes at different sites on the phosphorylated enzyme. However, no satisfactory explanation can be offered why the rate of reactivation by uncharged reactivators should be greatly increased in the presence of electrolytes.

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Isolation of 2-methoxyoestrone from the urine of pregnant women

KRAYCHY AND GALLAGHER[1] have recently reported the isolation of 2-methoxyoestrone from the urine of human subjects following the administration of oestradiol-17β-16 ^{14}C. This finding has been confirmed by ENGEL *et al.*[2]. In the course of our own studies on urinary oestrogen metabolites, the same compound has now been isolated from the urine of pregnant women, and observations of some interest have been made concerning its behaviour in the Kober reaction.

Preliminary experiments were carried out on synthetic 2-methoxyoestrone generously supplied by Dr. T. F. GALLAGHER of the Sloan-Kettering Institute, New York.