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Some species differences in the rates of reaction of diaphragm particulate acetylcholinesterases with tetraethyl pyrophosphate and pralidoxime

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PRETREATMENT of guinea pigs with non-toxic doses of TEPP (tetraethyl pyrophosphate) raised the LD_{50} of Soman (1,2,2-trimethylpropyl methylphosphonofluoridate) by a factor of about 7, provided that atropine and P2S (2-hydroxyiminomethyl-*N*-methylpyridinium methanesulphonate) were given shortly after Soman.[1] This treatment was ineffective in rats, 2 LD_{50}'s being fatal.[1] The postulated mechanism of action[1] is that TEPP inhibits most or all of the acetylcholinesterase (AChE; EC 3.1.1.7) of vital organs which is in excess of that required to support normal function, and that at least part of the TEPP-inhibited fraction is later reactivated by P2S. This simple hypothesis does not explain why the procedure is effective in the guinea pig but not in the rat, nor why TMB-4 (1,3-di(4-hydroxyiminomethylpyridinium) propane dihalide) given to guinea pigs instead of P2S is therapeutically ineffective in spite of being a better reactivator *in vitro*.

Parenteral administration of quaternary aldoximes fails adequately to reactivate brain AChE[2–4] because of poor penetration.[5,6] The action of these oximes is therefore mainly peripheral, and the diaphragm has been chosen as representing a vital peripheral organ.[7] Berry and Rutland[8] showed that there are two forms of AChE in diaphragm muscle of guinea pig or rat, a soluble form not sedimented by centrifuging at 100,000 *g* for 60 min, and a particulate form. The latter is almost certainly directly associated with neuromuscular function, and it is this fraction which has been studied. The "short method", by centrifuging at 500 *g*,[8] was used in the present study. The purpose of the experiments was to look for species differences in the kinetic properties of the particulate AChE, which might explain the observed toxicological findings.

The velocity of hydrolysis of 5·5 mM acetylcholine, in an initial volume of 10 cm^3 was measured by automatic continuous titration,[8] using a twin syringe assembly to keep the concentration of substrate constant. The general procedure was first to record a normal velocity, then to add 2–10 mm^3 of a stock solution of 1 mM-TEPP in propan-1-ol (to a final concentration of 0·2–1 μM). When a steady inhibited rate was established, 0·5 cm^3 of oxime solution was added, and recording continued until a new steady rate was noted. Control experiments showed that neither the dilution with titrants or oxime nor the propanol caused any appreciable departure from linearity in prolonged experiments.

It appeared that inhibition by TEPP was pseudo-reversible, i.e. that the inhibitor was stable during the period before adding oxime, and that at the steady state the rates of phosphorylation and dephosphorylation were equal. Rigorous proof of this hypothesis is difficult with preparations of low specific activity such as these.

Inhibition of guinea-pig preparations by TEPP in the range stated gave final steady rates of 15–20 per cent in 40–50 min. The final concentration of P2S then added was 0·1 mM, approximately that found in the diaphragm *in vivo* 30–60 min after intramuscular injection of the therapeutic dose of

30 mg/kg.[9] Reactivation to a new steady state in the region of 20–30 per cent of normal took 45–50 min. When concentrations of TMB-4 in the region of those found in the diaphragm *in vivo* after administration of therapeutic doses[10] were added, reactivation was so rapid that no transitional curve was seen between initial and final velocities.

Inhibition of rat preparations by the same concentrations of TEPP was extremely rapid, the transition curves being mostly too short for the estimation of rate constants. The magnitudes of the inhibitions were much the same as in the guinea pig. Addition of 0·1 mM-P2S gave extremely rapid reactivation, again to around 25 per cent.

These observations lead to the supposition that a major factor in the "protective" action of TEPP is the speed of the inhibition and reactivation processes. We found[1] that if TEPP were given to guinea pigs only 1 min before Soman the "protective" effect was little better than if only atropine and P2S were given, whereas the maximum "protection" was found if the interval between TEPP and Soman was 0·5–5 hr. This is evidently a reflexion of the relatively slow rate of inhibition of AChE by TEPP. P2S is an effective oxime in the guinea pig, possibly because the slow reactivation lags behind the clearance of Soman from the diaphragm, whereas it is ineffective in the rat because the rapid reactivation occurs while there is still enough free Soman present to re-inhibit the reactivated fraction of enzyme. Similarly rapid reactivation by TMB-4 explains its therapeutic ineffectiveness in the guinea pig when used in this manner. It is not yet possible to refine this hypothesis by studies of changes in the concentrations of free TEPP or Soman, because no method is known whereby the nanogram quantities of organophosphate can be estimated in tissues.

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Inhibition of guanine nucleotide biosynthesis by mycophenolic acid in Yoshida ascites cells

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Mycophenolic acid suppresses the growth of a wide range of transplantable tumours in rats and mice.[1,2] Studies on the mode of action of the compound indicate that its main inhibitory effect is against the biosynthesis of guanine nucleotides.[3] We found that mycophenolic acid is a very potent inhibitor of inosine monophosphate (IMP) dehydrogenase.[3] Since these mode of action studies involved the addition of mycophenolic acid to cells and enzyme preparations *in vitro* we thought it important to study the effects of orally administered mycophenolic acid on the fate of [^{14}C]hypoxan-