Penetration of oximes across the blood-brain barrier. A histochemical study of the cerebral cholinesterases reactivation[1]

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Summary. The comparison of the effects of 4 oximes upon the cerebral cholinesterases reactivation after intoxication with paraoxon shows that the best results are obtained with toxogonine and 1574 [(carbaldoxime-4 pyridinium)-1(methyl-1 imidazolium-3)-3 propane]. The reactivation power of this latter compound seems due to the ease with which it can pass through the blood-brain barrier.

Repeated administration of high doses of atropine in combination with oximes (e.g. pralidoxime or obidoxime) is the most widely used treatment against intoxication with organophosphates[2]. Oximes act as reactivators of cholinesterases, but the existence of a blood-brain barrier (BBB) against these compounds often prevents a good reactivation of the central specific cholinesterases[3-5]. We therefore started a comparative study of the cerebral cholinesterases reactivation, in vitro and in vivo, by different oximes, after inhibition with paraoxon.

The effects of the drugs (paraoxon and oximes: 2-PAM [pralidoxime], toxogonine [obidoxime], 1574 and 1405; see formulas in the table) were evaluated by the histochemical method of Koelle and Friedenwald[6], which permits the vizualization of the enzymatic activity of cholinesterases (incubation: 2 h at 37 °C; medium buffered at pH 6.0). This method was applied upon slices of the caudato-putamen (CP) from rats (Wistar origin); this structure is known to contain high levels of cholinesterases[7].

Results. 1. Inhibition experiments. The dose of paraoxon which gave a 'histochemically total' inhibition was determined in vitro and in vivo:

a) In vitro, cryostat slices of 18 µm from fresh tissue were stuck on glass cover-slips, dried, fixed for 10 min at room temperature in 10% neutral paraformaldehyde, rinsed and incubated for 10 min in solutions of paraoxon at various concentrations (from 10^{-9} to 10^{-1} M). The 'histochemically total' inhibition was observed for concentrations of paraoxon higher than 10^{-5} M (table).

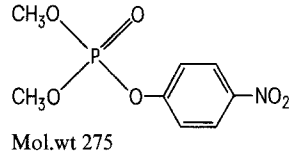
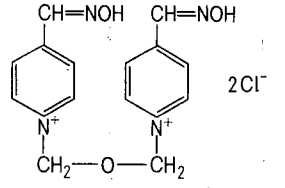
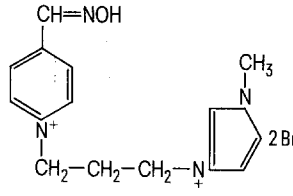
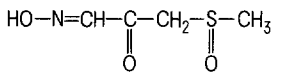
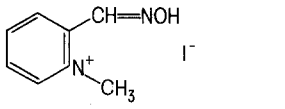
b) In vivo, paraoxon was administred by intracarotidian route in a dose range of 0.4–4 µM/kg b.wt. Total inhibition was observed after administration of doses higher than 1 µM/kg b.wt. This dose is less than the LD_{50} (2 µM/kg b.wt.)[8].

2. Reactivation experiments

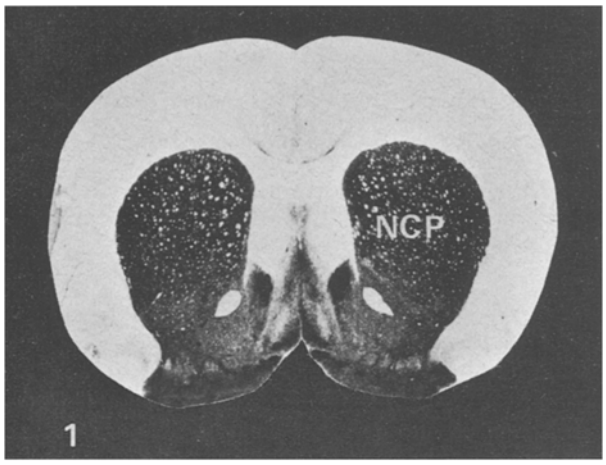
a) In vitro: For each oxime 5 rats were used, for which repeated experiments gave reproducible results. Slices were treated by 10^{-5} M paraoxon as described, and after extensive washing to remove the excess of organophosphate, they were treated for 10 min by oximes solutions at various concentrations. Enzymatic activity was revealed by Koelle's method after washing. Toxogonine was the best reactivator and was followed by 1574, 1405 and 2-PAM (table). It must be pointed out that after reactivation, the enzymatic activities were always lower than those in controls. Generally, the reactivation level increased with the oxime concentration. In the case of toxogonine, it reached a maximum and then decreased, perhaps because of an inhibitory effect of toxogonine at high concentration.

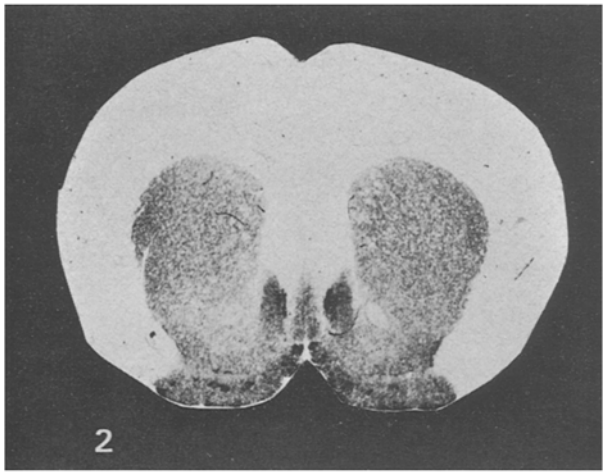
b) In vivo: Each oxime was tested in 50 animals and about 80% of our experiments gave consistent results. Paraoxon (1 µM/kg b.wt) and then 2 min later atropine (10 mg/kg b.wt) and 4 min later oxime (from 10 to 100 mg/kg b.wt) were injected by a heparinized catheter placed in the right common carotid. Atropine was used as a cholinolytic drug but had no direct effect on the cholinesterase activity. Animals were sacrified 10 min or 30 min after the first injection and slices of CP underwent the histochemical method (figure). As in vitro, the reactivation level increased with the oxime concentration, but at high concentrations, nonnegligeable percentage of death occurred, probably due to secondary effects of oximes. With nonlethal doses of 50 mg/kg b.wt the best reactivations were obtained with toxogonine and 1574, followed by 1405 and 2-PAM (table).

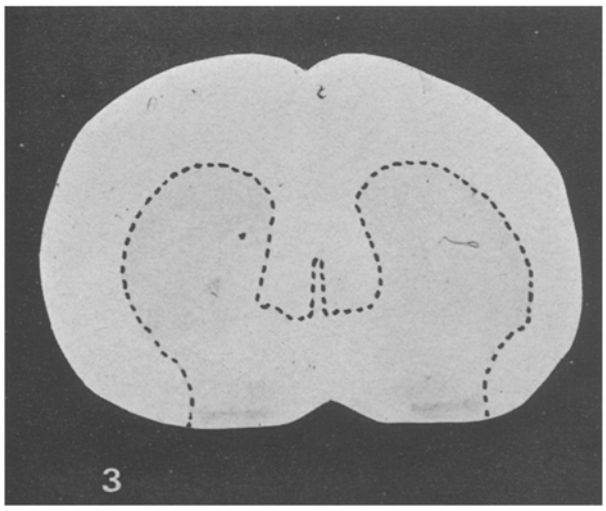
NCP cholinesterases inhibition by paraoxon and reactivation by oximes in vitro and in vivo

Drug's formulas	In vitro		In vivo
 Mol.wt 275 Paraoxon (E 600)	10^{-2} M	0	0
	10^{-3} M	0	(1 µM/kg b.wt)
	10^{-4} M	0	
	10^{-5} M	0	
	10^{-6} M	+	
	10^{-7} M	++	
	10^{-8} M	+++	
	10^{-9} M	++++	
 Mol.wt 359 Toxogonine	10^{-2} M	+	++
	10^{-3} M	+	(50 mg/kg b.wt)
	10^{-4} M	++	
	10^{-5} M	+++	
	10^{-6} M	++	
	10^{-7} M	+	
	10^{-8} M	0	
 Mol.wt 406 1574	10^{-2} M	+++	++
	10^{-3} M	++	(50 mg/kg b.wt)
	10^{-4} M	++	
	10^{-5} M	+	
	10^{-6} M	0	
 1405 Mol.wt 149	10^{-1} M	+++	+
	10^{-2} M	++	(50 mg/kg b.wt)
	10^{-3} M	+	
	10^{-4} M	0	
 Mol.wt 264 2-PAM	10^{-2} M	+	0+
	10^{-3} M	0	(50 mg/kg b.wt)
	10^{-4} M	0	

Normal activity: ++++. No detectable activity: 0.







NCP cholinesterases reactivation by toxogonine in vivo: 1: Control (normal animal). 2: Reactivation by 50 mg/kg b.wt toxogonine after inhibition by 1 μM/kg b.wt paraoxon. 3: Control (paraoxon only).

Better reactivations were observed when the animals were sacrified after 10 min than after 30 min; these results suggest that several phenomena may be implicated: inhibition of the reactivated enzyme by the free paraoxon, secondary inhibition by the phosphorylated oxime[8], or fast catabolism of the oxime compared to paraoxon.

Discussion. According to our results, the histochemical method of Koelle and Friedenwald appears as a sensitive and reproducible colorimetric method to compare the reactivation of the cholinesterases in vitro and in vivo by the different oximes after inhibition with paraoxon. In vitro, it can be assumed that in slices, numerous cellular membranes are broken, so that reagents are able to enter freely into cells; by this way, barrier problems are avoided, and it becomes possible to evaluate the intrinsic reactivating potency of the oximes, which is confirmed here. On the other hand, the efficiency of the reactivation of cholinesterases by oximes in vivo is very controversial in the literature[3,5]. The most discussed point is the penetration power of the drugs into the central nervous system. The present work shows that the oximes studied reactivate the cerebral cholinesterases to variable degrees and a good concordance was found between the results of the in vitro and in vivo experiments: Increasing reactivating power exists from 2-PAM to 1405, 1574 and toxogonine. This compound was in general the most potent in vitro, and thus it can be expected to show a strong reactivation in vivo. However, this was not found, and toxogonine gives the same range of reactivation as 1574. High concentration of toxogonine does not reactivate the cholinesterases well and even may have an inhibitory effect. Thus, in vivo the dose of 50 mg/kg b.wt toxogonine may give an inhibitory concentration in the brain: however, our preliminary studies have shown that 50 mg/kg b.wt seems to be an optimal dose for the reactivation of cerebral cholinesterases in rat, and using this dose, Erdmann[3] and Hobbiger et al.[5] demonstrated that only a minor quantity of toxogonine was found in the brain. Therefore, apparently toxogonine passes through the BBB less easily than other oximes, e.g. 1574, which is a moderate reactivator in vitro but which has a good reactivating power in vivo. Moreover, this last compound is also less toxic than toxogonine.

Finally there are 2 possibilities for the increase of reactivation of the cerebral cholinesterases: First the synthesis of new compounds having a good intrinsic reactivating potency and which easily pass the BBB. The second, in the case of powerful but toxic reactivators, the reversible lesion of the BBB is a way to increase their penetration and to lower their concentration and thus their toxic effect.

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