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Reactivation of acetylcholinesterase activity by two new oximes in diisopropylfluorophosphate-intoxicated mice

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Diisopropylfluorophosphate (0.5 LD₅₀, i.p.) significantly inhibited acetylcholinesterase (AChE) activity in blood and in cerebral cortex and corpus striatum of brain 1 h after administration in mice. Percentage reactivation of blood AChE was greater with pralidoxime than with the new oximes DEV-9 and DEV-10 at a dose of 50 mg/kg, i.p. along with atropine at 10 mg/kg, i.m. However, cortical AChE was reactivated with DEV-9 and DEV-10 without any effect on striatal AChE. We conclude that the protection afforded by oximes was due to peripheral reactivation of AChE.

It is widely accepted that most of the organophosphorus compounds inhibit the enzyme acetylcholinesterase (AChE), thereby causing toxic effects in humans and in animals¹. The phosphorylated (inhibited) enzyme can be reactivated by certain drugs (cholinesterase reactivators) such as hydroxylamine, hydroxamic acid and oximes². Among the oximes, pralidoxime, obidoxime and trimidoxime are well-known cholinesterase reactivators³. But the controversy on the cholinesterase reactivating drug which would be universally preferred is still open. In the present study we have evaluated reactivation of AChE by the oximes DEV-9 (1-[3-(hydroxyiminomethyl)-1-pyridino]-3-[4-carbamoyl-1-pyridino]-propane dibromide) and DEV-10 (1-[3-(hydroxyiminomethyl)-1-pyridino]-3-[3-(carboxymethyl)-1-pyridino]-propane dibromide) (Figure 1), synthesized by the condensation reaction of 1-bromo-3-[3-(hydroxyiminomethyl)-1-pyridino]-propane bromide with isonicotinamide and carboxymethylnicotinamide respectively as described by Sikder *et al.* (unpublished data), in diisopropylfluorophosphate (DFP)-treated mice.

Thirty male Swiss mice (20–25 g) were randomly

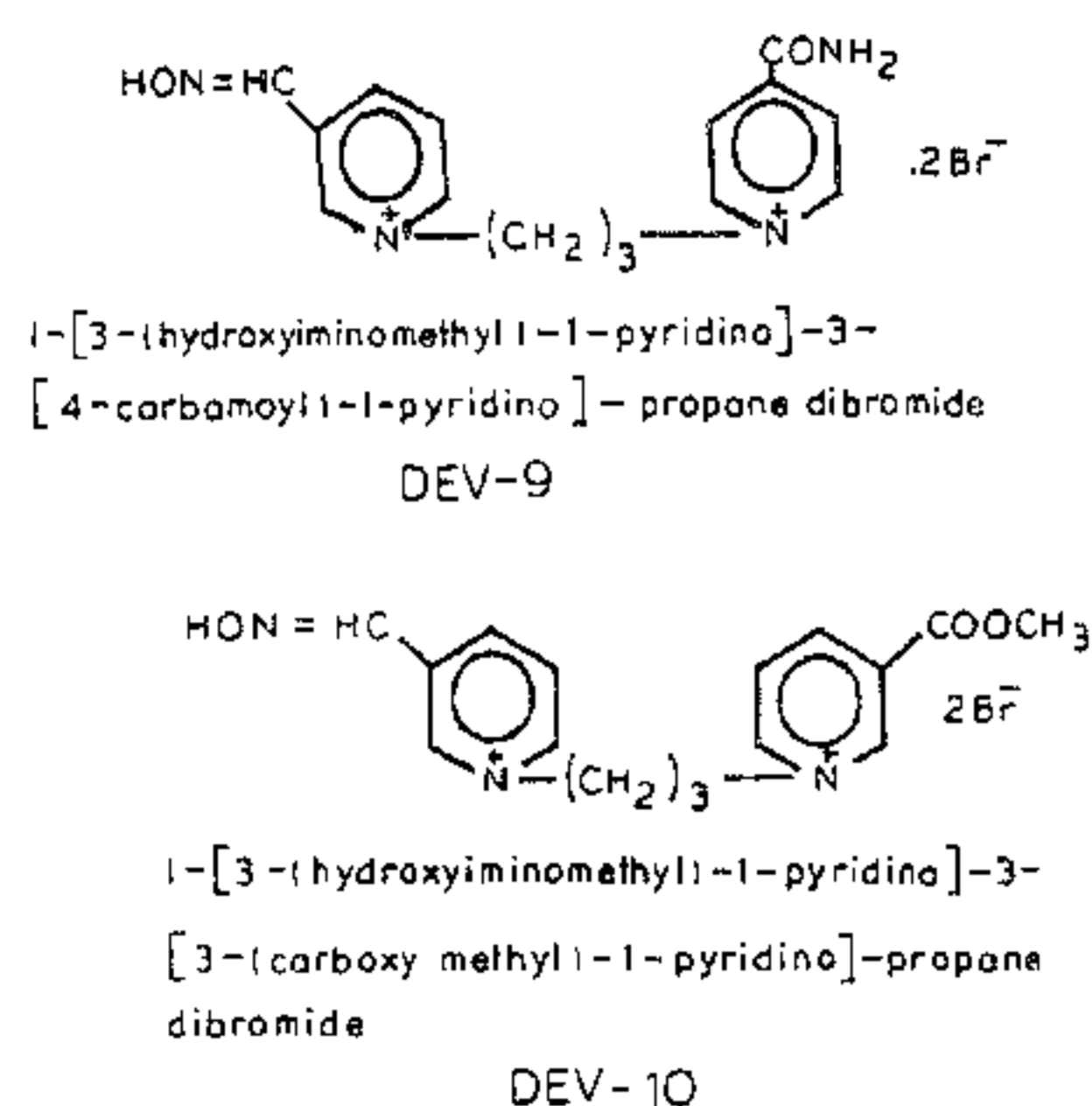


Figure 1. The new oximes DEV-9 and DEV-10.

divided into five groups of six animals each. They were provided food and water *ad libitum* except for a 12-h fasting before their use in the experiments. The animals in group 1 were treated with normal saline and served as control whereas those in group 2 were injected DFP (in normal saline) intraperitoneally (2 mg/kg = 0.5 LD₅₀). Animals in groups 3, 4 and 5 were first treated with DFP, then atropine (10 mg/kg, i.m.), followed immediately by respectively, pralidoxime (2-formyl-1-methylpyridinium oxime chloride, 2-PAM), DEV-9 and DEV-10, each at 50 mg/kg i.p. Atropine was given to protect the animals from the acute, lethal effects of DFP. Animals were sacrificed 1 h later and the blood was collected in heparinized tubes. Brain was dissected into two parts, cerebral cortex and corpus striatum, according to the method of Glowinski and Iverson⁴. Blood and brain tissue AChE activity was assayed as described by Ellman *et al.*⁵ using acetylthiocholine as substrate. Protection index (PI) was determined by the method of Das Gupta *et al.*⁶ Data were analysed statistically using Student's *t* test.

The inhibition of AChE in blood was 84% while in cortex and striatum it was 66% and 76% respectively 1 h after DFP administration (Table 1), indicating more binding of DFP to blood AChE and penetration into deeper structures of the brain. However, the inhibition was more in striatum than in cortex. It is suggested that even a small change in AChE activity can be detected in this brain region, which has been reported to be involved in the control of motor activity⁷ and also rich in AChE enzyme⁸. Further the results indicate that all three oximes significantly reactivated AChE inhibited by DFP in the blood but DEV-9 and DEV-10 did so to a smaller extent than 2-PAM. However, 2-PAM failed to reactivate DFP-inhibited cortical and striatal AChE activity, while DEV-9 and DEV-10 both reactivated cortical but not striatal AChE activity (Table 1). It has been previously reported that pyridine aldoximes have the disadvantage of limited tissue distribution. Being

Table 1. Effect of oximes on AChE activity in DFP-treated mice.

Group	AChE activity*			Protection index
	Blood	Cerebral cortex	Corpus striatum	
1. Control	2.86 ± 0.40	5.96 ± 0.42	20.45 ± 0.88	—
2. DFP	0.47 ± 0.10 ^a	2.01 ± 0.28 ^a	4.85 ± 0.48 ^a	—
(% inhibition)	84.0	66.0	76.0	—
3. DFP + atropine + 2-PAM	2.60 ± 0.24 ^b	2.08 ± 0.26	4.76 ± 0.41	4.40
(% Reactivation)	89.0	1.80	00	
4. DFP + atropine + DEV-9	2.39 ± 0.20 ^b	2.48 ± 0.32 ^c	4.80 ± 0.50	3.50
(% Reactivation)	80.0	12.0	00	
5. DFP + atropine + DEV-10	2.30 ± 0.22 ^b	2.40 ± 0.30 ^c	4.99 ± 0.56	4.50
(% Reactivation)	77.0	10.0	00	

Each value is mean ± SE of six animals.

*Activity expressed as μ moles acetylthiocholine hydrolysed per min per g tissue or ml blood.

^aP < 0.001, vs group 1; ^bP < 0.001, vs group 2; ^cP < 0.05, vs group 3.

quaternary salts, these compounds do not readily penetrate into the central nervous system, causing poor reactivation in cerebral tissues⁹. Our results indicate that DEV-9 and DEV-10 partially crossed the blood-brain barrier and reactivated cortical AChE. Since, structurally the blood-brain barrier is different from area to area of the brain¹⁰, reactivation of striatal (deeper area) AChE was not observed (Table 1). Protection studies indicated that both DEV-9 and DEV-10 gave appreciable protection, like 2-PAM, in DFP poisoning. We conclude that the protection afforded by the oximes was mainly due to peripheral reactivation of AChE. The efficacy of 2-PAM was more than that of the new oximes.

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Immobilization of α -amylase complex in detection of higher oligosaccharides on paper

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The method of identification of high-molecular-weight oligosaccharides on paper is limited in its sensitivity by the silver nitrate test. A method described here generates more reducing power of higher sugars *in situ*, facilitating easy identification of the spots. Chromatograms dipped in 0.1% of Sanzyme solution in 80% acetone adsorb α -amylase. After removal of acetone, enzyme was allowed to react on paper for 6 h in a moist chamber. This degrades high-molecular-weight sugars, generating higher reducing power. Consequently, they are easily detected on the paper by the silver nitrate test. It is a sensitive method of identification of higher oligosaccharides which could be routinely used in the study of natural products.

PAPER chromatography is a rapid, sensitive and inexpensive procedure in detection of low-molecular-weight biological molecules, especially carbohydrates. In conventional methods of separation of sugars, a sample mixture is applied at the origin of chromatographic paper, developed in a suitable solvent, and the spots are detected by silver nitrate test¹. The reducing sugars appear as distinct spots, and the intensity of spots are dependent upon the amount of sample and reducing power of sugars. However, this procedure is limited to a few low-molecular-weight sugars. The high-molecular-weight compounds escape unnoticed as they possess less reducing power, although they contain considerable amounts of carbohydrate. In this communication, a procedure is described for identification of high-molecular-weight sugars on paper using an α -amylase complex. After separation of sugars, the reducing power of high-molecular-weight compounds is increased by adsorbing α -amylase complex on the paper and allowing the enzyme to act till the separated spots undergo further degradation *in situ*, generating more reducing power.

α -Amylase complex is commercially available in a powder form as Sanzyme, Uni-Sankyo Limited, Hyderabad. It is a crude fungal enzyme prepared by ethanol extraction of *Aspergillus oryzae*. It is reported to contain essentially α -amylase activity associated with another saccharogenic enzyme Taka-amylase B which converts starch into glucose.

Incomplete starch hydrolysates were prepared by reacting Sanzyme with 2% soluble starch solutions. One gram of starch was dispersed and gelatinized in 40 ml of distilled water. To it 40 mg of Sanzyme