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The effect of organophosphate poisoning on plasma cyclic AMP in rats

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Cyclic adenosine monophosphate which is known as molecule confined to regulation of protein kinase activity, is present in all types of mammalian cells. It is generated via activation of intracellular adenylate cyclase and released to the extracellular fluid [1–7]. Cyclic nucleotide change in the plasma and urine has been reported to occur in response to various hormonal deficiences and treatments [8–11]. Thus, the increase in plasma cAMP was found to follow stimulation of β-adrenergic receptors by endogenous catecholamines [11] as well as administration of glucagon and isoprenaline [6]. Dramatic increase in plasma cAMP following poisoning with soman, has been revealed by Stitcher *et al.* [12] and interpreted as to be caused by the ACh-induced release of humoral and pharmacologically active substances which induce cAMP-generation; the cAMP participates in anti-ChE poisoning by enhancing the concentration of ACh in the brain or by enhancing the release of ACh at the neuromuscular junctions [13].

While the role played by cAMP in anti-ChE poisoning is within the scope of speculation, the plasma cAMP, being in a dynamic steady-state relation with its intracellular pools [7], could be used for assessment of the effect of poisoning on the cAMP-mediated cellular processes. In this context we studied the dependence of plasma cAMP level on the type and dose of organophosphate along with the effect of antidote on the restoration of the poison-induced rise in plasma cAMP and inhibition of RBC AChE activity.

Male albino rats, weighing 190–220 g were used after a 18–20 hr fast. Animals were poisoned by subcutaneous administration of 0.9–1.2 LD_{50} soman or 0.3–1.2 LD_{50} VX. At the onset of convulsions, 10–15 min after poisoning, one out of four animal subgroups received saline, whereas the others received intramuscular injections of (1) HI-6, 50 mg/kg, (2) atropin, 10 mg/kg, (3) the mixture of atropin 10 mg/kg and HI-6,5/mg/kg. The same number of non-poisoned, control animals were divided into four subgroups and treated according to the same protocol as the poisoned ones.

Untreated or saline treated rats were decapitated at the onset of convulsion phase, 10–15 min following poisoning, the others, 1 hr after HI-6 or atropin administration. Blood aliquots (2 ml) were collected in centrifuge tubes containing 20 μl of 0.5 M EDTA, pH 7.5 and centrifuged. The cAMP was determined in blood plasma by radioimmuno assay method. The cAMP assay kit was Radiochemical Centre (Amersham, U.K.) product. The AChE activity in erythrocytes was estimated by Michel method [23] using acetyl-β methylcholine chloride as substrate.

In plasma of male rats the cAMP level was approx 20 pmol/ml (Table 1), whereas in females the concentrations were two-fold higher (data not shown). Depending on sex of animals, the reported values ranged from 14 to 37 pmol/ml [11, 14, 18]. Thus, in plasma of female rats Patterson *et al.* [18] found 37 pmol/ml, while for the male rats Sarkar *et al.* [15] and Turinski [14] reported 14 and 20 pmol/ml, respectively. Circadian variations of plasma cAMP, which has been demonstrated to exist [19] may also contribute to a large variations of normal values.

Abbreviations used: cAMP, cyclic adenosine monophosphate; ACh, acetylcholine; AChE, acetylcholinesterase (E.C.3.1.1.7); RBC, red blood cells; VX, O-ethyl-S-(2-diisopropylaminoethyl)methylphosphotioate; soman, pinacolyl methylphosphonofluoridate; HI-6,1-(2-hydroxyiminomethyl-1-pyridinio-3-(4-carbamoyl-1-pyridinio)-2oxapropane dichloride.

Table 1. Concentration of plasma cAMP following organophosphates poisoning and antidote therapy (mean values ± S.E.)

Poisoning	cAMP (pmol/ml)			
	Control	HI-6*	Atropin*	HI-6+Atropin
None	19.8 ± 1.1 (47)	30.5 ± 2.5 (15)	15.5 ± 1.3 (12)	21.0 ± 2.1 (12)
1.2 LD_{50} Soman	111.7 ± 6.2 (30)	103 ± 16.3 (10)	30.8 ± 1.6 (4)	37.0 ± 9.0 (5)
1.2 LD_{50} VX	152.0 ± 16.8 (31)	40.5 ± 5.2 (18)	33.5 (2)	24.7 (3)

* Rats received: HI-6, 50 mg/kg, atropin sulphate, 10 mg/kg at the start of convulsions (10–15 min after 1.2 LD_{50} soman or VX). They were killed 60 min later. Figures in parentheses refer to the number of animals used in experiments.

Table 1 shows that administration of the bisquaternary monooxime HI-6 into rats caused a moderate, but statistically significant, raise in plasma cAMP, while atropin treatment had an opposite effect. When the mixture containing both substances was administered, the level of plasma cAMP remained unchanged.

Injection of 1.2 LD_{50} soman or VX was lethal and followed by convulsions which appeared 10–15 min after administration of poison and parallelled the increase of plasma cAMP. Table 1 shows that in plasma of rats poisoned with soman and VX the cAMP rose to 111 pmol/ml and to 157 pmol/ml, respectively. The HI-6, when administrated at the beginning of the convulsion phase, caused the recovery and survival in both the soman and the VX-intoxicated rats. The recovery coincided both with the reactivation of RBC AChE and the normalization of cAMP in plasma of VX-intoxicated rats only, whereas in soman-poisoned rats neither AChE reactivation, nor cAMP decrease was found to occur one hour after HI-6 administration.

The lack of correlation between the concentration of cAMP in plasma and the survival of animals was confirmed also in experiments where convulsions were depressed by atropin. Table 1 shows that atropin treatment of rats poisoned either with soman or VX resulted in an almost complete restoration of normal cAMP concentration in plasma. Despite this, most animals died.

The dependence of plasma cAMP and AChE activity on VX dose and HI-6 treatment was investigated in greater detail in experiments where increasing doses of VX were injected into rats and RBC AChE activity and plasma cAMP were determined prior to and after HI-6 administration. Results presented in Fig. 1 show that rising doses of VX caused a sigmoidal increase of plasma cAMP and a parabolic decrease of RBC activity [Fig. 1(A)]. Administration of HI-6 resulted in a substantial decrease of plasma cAMP and reactivation of RBC AChE [Fig. 1(B)].

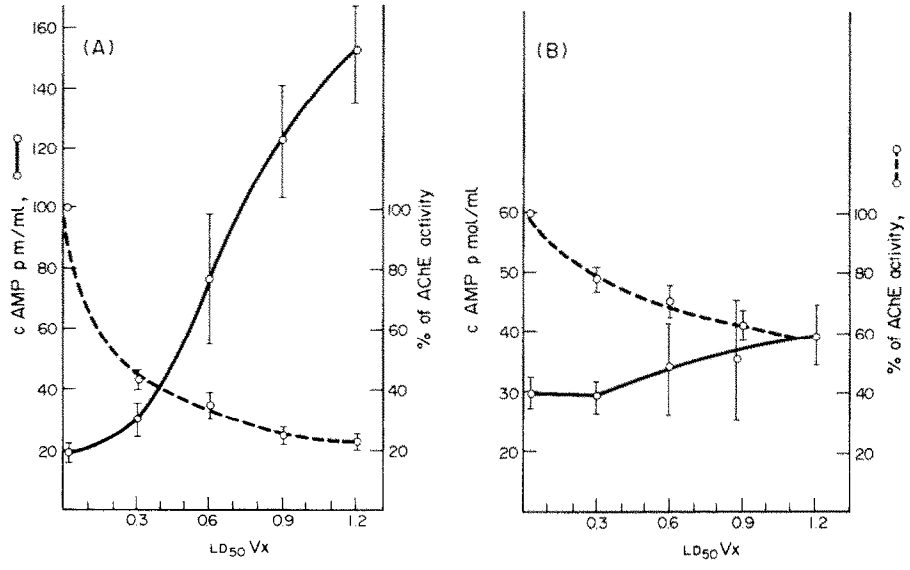

Fig. 1. cAMP content in plasma and AChE activity in erythrocytes of rats receiving VX.(A) Dose–response curves following subcutaneous injection of various doses of VX. (B) Dose–response curves 1 hr after HI-6.

Table 2. The effect of VX on plasma cAMP in rats pretreated with reserpine

	Untreated	Reserpine pretreated		
		Control	1.2 LD_{50}	HI-6 therapy
cAMP pmol/ml	19.8 ± 1.1 (47)	21.1 ± 2.6 (5)	69.5 ± 12.0 (11)	36.7 ± 11.3 (4)

Animals were treated twice daily with reserpine (2.5 mg/kg, s.c.) for 2 days prior to poisoning. Figures in parentheses refer to the number of animals used in experiments.

To assess whether the endogenous catecholamines influence the VX-induced increase in plasma cAMP, the cellular pools were depleted by treating animals with reserpine prior to VX administration. Table 2 shows that reserpine pretreatment, while failing to affect concentration of cAMP in plasma of control animals, diminished considerably the cAMP increase in plasma of poisoned animals.

The results presented in this report showed that inhibition of AChE in soman- and VX-poisoning coincided with a significant increase of plasma cAMP. while administration of HI-6 provided a full protection in poisoning with otherwise lethal doses of 1.2 LD_{50} soman and VX, its effect on restoration of plasma cAMP and RBC AChE activity was found to be poison-dependent. In VX-intoxication HI-6 reactivated RBC AChE and remarkably reduced the level of cAMP in plasma, whereas in soman-poisoning HI-6 administration caused neither RBC AChE reactivation, nor cAMP decrease. The revealed RBC AChE non-reactivating effect of HI-6, would suggest that recovery in soman poisoning resulted from the HI-6 activity which is not AChE-related. It seems more likely, however, that HI-6, though failing to reactivate RBC AChE, did reactivate AChE at vital, for a survival essential, sites of the body, as it has been demonstrated by van der Meer and Wolthuis [20] and by Wolthuis and Kepner [21]. Such an interpretation is, also, in agreement with the assumption that therapeutic effect of HI-6 in soman poisoning is based on AChE reactivation [22].

The correlative relationship between the concentration of plasma cAMP and the RBC AChE activity in the VX-poisoned rats suggests that mechanisms regulating the cAMP level in plasma and those regulating AChE activity are linked. Depletion of cellular catecholamines by reserpine was found to reduce remarkably the VX-induced increase of plasma cAMP. The cAMP generated via catecholamines activation of adenylate cyclase could be, hence, speculated to participate in the VX-induced increase of plasma cAMP. In view that ACh has been proposed to induce catecholamines release in soman intoxication [13], it seems likely that ACh is the key molecule which interrelates the AChE inhibition and the cAMP generation processes. The effect of poisoning with soman and VX as well as those of HI-6 therapy on the concentration of cAMP in plasma were investigated and related to alterations in RBC AChE activity. Soman and VX were found to cause a many fold increase in plasma cAMP. HI-6 therapy, though effective in both intoxications, led to restoration of cAMP concentration and AChE activity in VX poisoning, but not in poisoning with soman.

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