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Toxicologic Significance of the Hyperglycemia Caused by Organophosphorous Insecticides

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Hyperglycemia is one of the side-effects in poisonings by organophosphorous (OP) insecticides in humans with blood glucose rising up to five fold (Hayes et al. 1978; Meller et al. 1981; Namba et al. 1971). The ability of OPs to cause hyperglycemia has been confirmed in the laboratory animals (Dybing and Sognen 1958; Fletcher et al. 1988; Matin and Siddiqui 1982). The mechanism of OP-induced hyperglycemia has not yet been identified but it is speculated to be due to OP inhibition of acetylcholinesterase(s) of the central or peripheral synapses that act in the endocrine regulation of glucose metabolism (Kant et al. 1988; Matin and Siddiqui 1982). Stress as a consequence of the poisoning by OPs is perhaps an additional aggravating element (Clement 1985; Fletcher et al. 1988; Kant et al. 1988).

In this study we investigated the conditions that result in OP-induced hyperglycemia with the objective to assess its toxicological significance. We have also examined our hypothesis, whether an increase in diabetogenic (Kotake and Inada 1953) xanthurenic acid caused by some OP insecticides (Pewnim and Seifert 1993; Seifert and Pewnim 1992) contributes to OP-induced hyperglycemia.

MATERIALS AND METHODS

Diazinon [O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate; Ciba Geigy, >99%] was used as a model OP compound because of its ability to cause hyperglycemia in both humans and animals (Dybing and Sognen 1958; Namba et al. 1971) and to increase formation of xanthurenic acid in mice (Seifert and Pewnim 1992). Its purity was checked periodically by thin layer chromatography and by gas chromatography-mass spectrometry.

Mice (Swiss albino) were purchased from Simonsen (Gilroy, CA) and Charles River Laboratories (Massachussetts). Male mice 4-8 weeks old were used throughout this study. They were kept on a 12-hour light-darkness cycle with free access to the diet (Purina Chow) and water except for the experiments where effects of diet restriction were investigated. Diazinon dissolved in 20 µl of triethylene glycol dimethyl ether was administered intraperitoneally to mice. A

standard dose of 150 mg of diazinon per kg of body weight used in most experiments was based on a dose-response study. This dose was $\leq LD_{10}$ and caused minimal symptoms of cholinergic toxicity. Activities of whole brain acetylcholinesterases (% of control ± S.D.) assayed with acetylthiocholine (Ellman et al 1961) were 83±19, 67±24, 75±4, 61±33 and 56±12 at 2 hr, 2, 6, 13 and 16 days, respectively, after diazinon administration. Typically a group of five mice was used for studying one specific parameter. Control mice were injected with the carrier solvent.

Time course of changes in blood glucose was measured from 0.5 hour up to 22 days after administration of a single dose of diazinon. The dose response was determined 1.5 hours after administration of 20 to 250 mg of diazinon per kg to mice; the latter dose was approximately the LD_{50}. The effect of diet availability on OP-induced hyperglycemia was studied in mice deprived of their diet for 24 hours before diazinon administration. The diurnal response of blood glucose to diazinon was investigated 1.5 hours after administering diazinon to mice at 1:00, 8:00, 14:00 and 20:00 hours.

The role of the pancreatic β-cells in OP-induced hyperglycemia was examined in a comparative study using mice whose pancreatic β-cells were destroyed by intraperitoneal administration of 200 mg alloxan per kg (Mordes and Rossini 1994). Diazinon was administered 3-4 days after alloxan treatment at the time of the maximal development of hyperglycemia. Blood glucose was assayed 1.5 hour later.

The effects of xanthurenic acid and two nonphosphorous insecticides carbaryl and endrin were investigated in mice from 1 hour to 10 days after xanthurenic acid intraperitoneal administration (0.1-20 mg/kg) and two hours after the administration of carbaryl and endrin. The effects of two OP antidotes, atropine (40 mg/kg) and 2-PAM (pyridine-2-aldoxime methiodide, 1, 10, and 100 mg/kg) on blood glucose were tested in mice both treated and untreated with diazinon. Atropine and/or 2-PAM were administered intraperitoneally to mice 10 min prior diazinon treatment.

Glucose was assayed enzymatically by coupled reactions using hexokinase and glucose-6-phosphate dehydrogenase (Kunst et al. 1984). Blood was collected from the thorax cavity or by tail bleeding 1.5-2.5 hours after diazinon administration except for the time course study. Blood was mixed with 0.33 M perchloric acid (1:3, w/v), centrifuged for 15 min at 3,000xg at 4°C and the supernatant used for the assay.

RESULTS AND DISCUSSION

Blood glucose increased up to 207±51% (S.D.) reaching 324% within 1-3 hours after diazinon administration (Fig. 1). The threshold dose of diazinon that caused

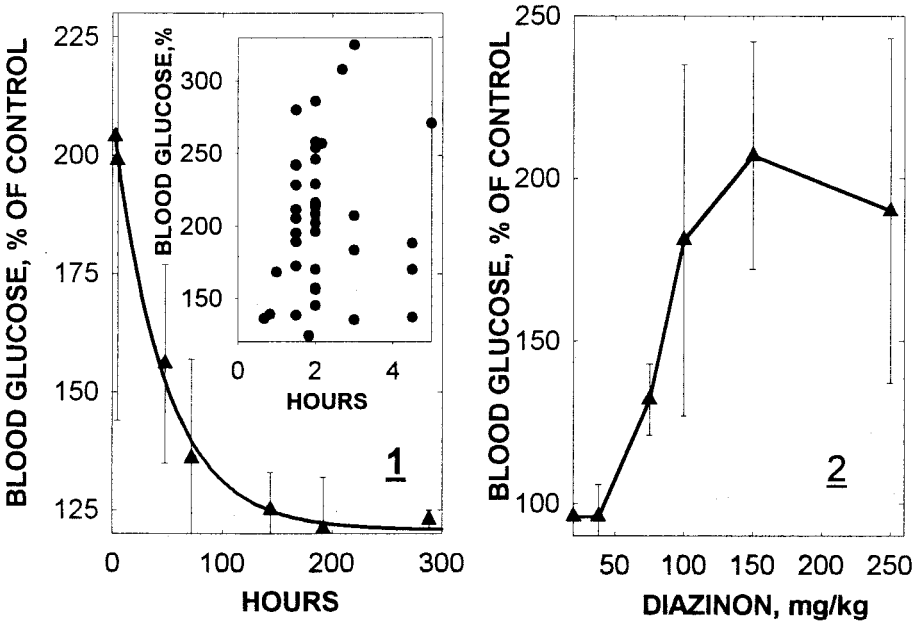

Figure 1. Changes in blood glucose after an intraperitoneal administration of diazinon (150 mg/kg). The spontaneous return to the basal values proceeded according to the equation $y=y_0+ae^{-bt}+ce^{-dt}$ (SigmaPlot). Inset is a detailed time course for the initial period. The standard deviations were calculated from blood glucose of five mice per time measured except for the value at 3 hours (n=38, calculated from glucose concentrations measured at 0.8 to 3 hrs). The mean blood glucose of control mice assayed over a period of two years was 136±37 (S.D.) mg glucose/100 g blood (n=183). Coefficient of variation of control blood glucose was ±18% in a standard assay using five mice.

Figure 2. Dose-response of mouse blood glucose related to diazinon. The standard deviations were calculated from blood glucose of five mice except for the value at 150 mg/kg (n=25).

hyperglycemia was 75 mg/kg (Fig. 2). Glucose levels peaked at approximately 200% of control above 100 mg/kg diazinon. The need for high OP insecticide doses for the appearance of hyperglycemia in mice and rats (Fig. 2) (Dybing and Sognen 1958; Matin and Siddiqui 1982) is similar with the doses found for human poisonings (Hayes et al. 1978; Meller et al. 1981; Namba et al. 1971). This finding suggests that OP-induced hyperglycemia has the potential to occur only in accidental or suicidal poisonings but not in dietary exposures to minute quantities of OP insecticides. Desensitization of blood glucose response to the multiple administration of an OP reported by Peoples et al. (1988) reinforces this conclusion.

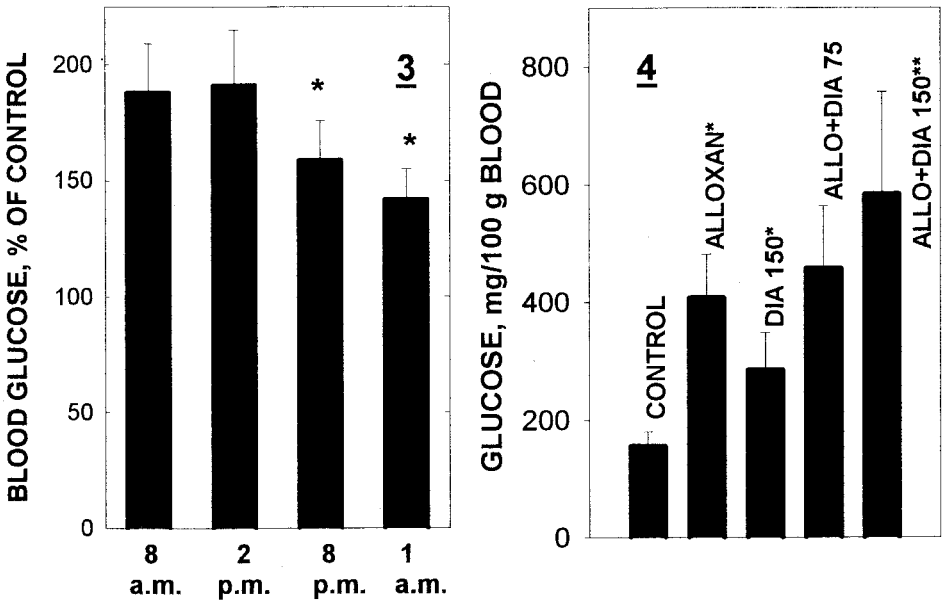

Figure 3. The effects of the time of diazinon administration on the increase in mouse blood glucose. Five mice were used per each assay. *Significantly different from the diazinon-increased blood glucose at 8 a.m., $p< 0.1$.

Figure 4. The increase in blood glucose in alloxan, diazinon (DIA 150, 150 mg/kg) and/or alloxan-diazinon-treated mice (ALLO+DIA 75, 75 mg diazinon/kg; ALLO+DIA 150, 150 mg diazinon/kg). Five mice were used per each treatment. *Significantly different from the control mice, $p<0.01$; **significantly different from the alloxan-treated mice, $p<0.05$.

Elevated blood glucose occurred in 52±12% (S.D., n=52) of the mice treated with diazinon (150 mg/kg). Due to the severe cholinergic toxicity at the highest diazinon dose tested (250 mg/kg) it was not feasible to determine whether this limited response reflected the normal distribution of mice population with regard to their susceptibility to OP-induced hyperglycemia or whether it indicated that approximately half of mice was resistant to this phenomenon. The dietary regimen did not affect the extent of OP-induced hyperglycemia. Blood glucose increased to similar levels after diazinon administration (204±57%, S.D., n=5) in mice with restricted access to the diet as in mice fed *ad libitum*. Blood glucose concentrations varied with the time of diazinon administration. Blood glucose was higher in mice treated with diazinon during the day than at night (Fig. 3). The diurnal variation of diazinon-elevated blood glucose is consistent with the speculation that the OP-induced hyperglycemia is mediated via a complex diurnal neuroendocrine system (Clement 1985; Fletcher et al. 1988; Kant et al. 1988).

Blood glucose increased even more after administration of diazinon to mice that were hyperglycemic after alloxan treatment (Fig. 4). This finding suggests that integrity of the pancreatic β-cells and an associated insulin production are not critical for the initiation of hyperglycemia by diazinon.

Our hypothesis that a direct insult of xanthurenic acid on insulin availability may cause the acute OP-induced hyperglycemia can be excluded based on three findings: First, the increase in xanthurenic acid due to diazinon peaked 20 hours after its administration to mice (Seifert and Pewnim, 1992) in contrast to the fast rise of blood glucose (Fig. 1). Second, the threshold dose of diazinon for raising xanthurenic acid (Seifert and Pewnim, 1992) was lower than the dose needed for an elevation of blood glucose (10 mg vs. 75 mg per kg). Third, the increase in blood glucose in mice treated intraperitoneally with xanthurenic acid was marginal (Table 1).

Table 1. Effects of atropine, carbaryl, diazinon, endrin, 2-PAM and xanthurenic acid on blood glucose

	Dose mg/kg	Blood glucose % of control
Diazinon	150	182±27
Atropine	40	201±40
2-PAM	100	116±14[a]
Diazinon+2-PAM	150+1	164±22
	150+10	124±21[a]
	150+100	102±11[a]
Diazinon+2-PAM+ +atropine	150+100+40	248±18
Carbaryl	100	143±34[b]
Endrin	80	138±12[b]
Xanthurenic acid	20 (1-5 hrs)	123±14[b]
	(1-5 days)	105±9

The values of the controls, diazinon-treated and diazinon/2-PAM-treated were obtained from ten mice, the remaining values from five mice, respectively. [a]Significantly different from the diazinon-treated mice ($p<0.05$); [b]significantly different from the control ($p<0.2$).

The correlation between the sizes of OP doses that elevated blood glucose and their respective LD_{50}'s supports the hypothesis that acetylcholinesterase is involved in OP-induced hyperglycemia. For instance, only 60 μg/kg of the extremely toxic OP soman (LD_{50}~80 μg/kg) (Fletcher et al. 1988) were needed to cause hyperglycemia in mice in contrast to 40-75 mg/kg of a moderately toxic diazinon (LD_{50}~250 mg/kg) (this study and Matin et al. 1987) and 500 mg/kg of the less toxic malathion (Matin and Siddiqui, 1982). Carbaryl, a moderately toxic anticholinesterase insecticide, only moderately raised blood glucose (Table 1).

More supporting evidence for a relationship between acetylcholinesterase inhibition and OP-induced hyperglycemia came from examination of two OP antidotes. 2-PAM, a reactivator of phosphorylated acetylcholinesterases, alleviated the diazinon-induced hyperglycemia at 10 to 100 mg/kg (Table 1). Atropine, an agent that acts on muscarinic receptors, caused hyperglycemia. It was ineffective in alleviating blood glucose even in a mixture with 2-PAM (Table 1). A chlorinated cyclodiene insecticide endrin only moderately elevated blood glucose (Table 1) even at doses that caused severe toxic symptoms unrelated to cholinergic toxicity.

A limitation of the toxicological significance of OP-induced hyperglycemia for the acute OP poisoning of humans and animals is that high blood glucose concentrations in acute OP poisonings recede spontaneously to normal level in healthy individuals. However, potentially severe consequences of OP-induced hyperglycemia may occur in diabetics where an additional increase in blood glucose due to OP's would further aggravate their health status.

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