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Insights into the mechanisms mediating hyperglycemic and stressogenic outcomes in rats treated with monocrotophos, an organophosphorus insecticide

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ABSTRACT

The present investigation provides mechanistic insights into the hyperglycemic and stressogenic effects of monocrotophos, an organophosphorus insecticide. Pre-treatment of rats with mifepristone (glucocorticoid receptor antagonist) prevented induction of liver tyrosine aminotransferase activity (TAT), but was ineffective in attenuating hyperglycemia induced by monocrotophos. Pre-treatment with propranolol (β-adrenergic receptor antagonist) and phentolamine (α-adrenergic receptor antagonist) were effective in abrogating monocrotophos-induced hyperglycemia. Interestingly, while propranolol offered partial protection against hyperglycemia, phentolamine completely abolished the same. However, monocrotophos-induced hyperlactacidemia was completely abolished by propranolol. Both the adrenoreceptor antagonists, however, failed to attenuate the stressogenic potential of monocrotophos. Hyperglycemia and hyperlactacidemia induced by monocrotophos were abolished by pre-treatment with atropine. Exogenous epinephrine was associated with hyperglycemia and hyperlactacidemia. The impact of adrenergic antagonists on epinephrine-induced hyperglycemia and hyperlactacidemia were remarkably similar to that of monocrotophos-induced hyperglycemia and hyperlactacidemia. Further, hydrazine sulfate (a gluconeogenesis inhibitor) abolished hyperglycemia in monocrotophos-treated rats. From our data, it can be hypothesized that excessive stimulation of adrenoreceptors, probably elicited by increased plasma epinephrine, mediates hyperglycemic outcomes induced by monocrotophos. Pattern of changes in plasma lactate suggests that β-adrenergic activation mediates monocrotophos-induced hyperlactacidemia, while α-adrenergic receptor mediates lactate utilization, leading to hyperglycemia. Induction of liver TAT activity is attributable to glucocorticoid receptor activation as a result of hypercorticosteronemia.

1. Introduction

Organophosphorus insecticides (OP) constitute one of the most widely used classes of pesticides in agricultural and household scenario. The primary toxicity of OP is a consequence of inhibition of acetylcholinesterase (AChE; EC 3.1.1.7), an enzyme involved in regulation of neurotransmission by hydrolysis of the neurotransmitter, acetylcholine (ACh). The acute toxicity of OP is due to cholinergic stress as a consequence of ACh accumulation and ACh receptor overstimulation (Sogorb and Vilanova, 2002; Abou-Donia, 2003). Hyperglycemia is now being increasingly reported as a manifestation of acute toxicity of OP (Rodrigues et al., 1986; Matin et al., 1989; Seifert, 2001; Lasram et al., 2008; Joshi and Rajini, 2009). In addition, OP and other AChE-inhibiting organophosphates have been demonstrated to interfere with hypothalamus–pituitary–adrenal (HPA) axis function as characterized hypercorticosteronemia (Osicka-Kaprowska et al., 1984; Smallridge et al., 1991; Kassa and Bajgar, 1995; Spassova et al., 2000; Joshi and Rajini, 2009) and increased activity liver gluconeogenesis enzymes (Matin et al., 1989; Abdollahi et al., 2004), including tyrosine aminotransferase (TAT) (Kassa and Bajgar, 1995; Joshi and Rajini, 2009).

Glucocorticoid hormones mediate crucial physiological processes such as glucose homeostasis and various stress responses (Baxter and Rosseau, 1979). Glucocorticoids (GC) facilitate gluconeogenesis in hepatocytes by transcriptional activation of gluconeogenesis enzymes. In view of the crucial role played by GC in glucose homeostasis, it becomes imperative to investigate the role of GC-signaling in OP-induced hyperglycemia. Epinephrine, an amine hormone, secreted by adrenal medulla plays an important role in carbohydrate metabolism. Since epinephrine is also known to induce hyperglycemia (Bloom and Russel, 1955; Yajima and Ui, 1974) and epinephrine secretion by adrenals is under cholinergic control, epinephrine signaling also becomes an attractive target in dissecting the mechanism of OP-induced hyperglycemia.

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Table 1
Summary of experimental protocols.

Expt. No.	Test compound	Pharmacological compound	Rationale of experiment	Endpoints
I	Monocrotophos	Mifepristone (glucocorticoid receptor antagonist)	To investigate the role of glucocorticoid signaling in hyperglycemic and stressogenic potential of monocrotophos	Blood glucose, plasma corticosterone and liver TAT activity
II	Monocrotophos	Phentolamine (α-adrenergic antagonist)	To investigate the role of α-adrenergic mechanisms in the ability of monocrotophos to induce hyperglycemia, physiological stress and hyperlactacidemia	Blood glucose, plasma corticosterone, plasma lactate and liver TAT activity
III	Monocrotophos	Propranolol (β -adrenergic antagonist)	To investigate the role of β-adrenergic mechanisms in the ability of monocrotophos to induce hyperglycemia, physiological stress and hyperlactacidemia	Blood glucose, plasma corticosterone, plasma lactate and liver TAT activity
IV	Monocrotophos	Atropine (muscarinic cholinergic receptor antagonist)	To investigate the role of cholinergic stress in the ability of monocrotophos to induce hyperglycemia and hyperlactacidemia	Blood glucose and plasma lactate
V	Epinephrine	–	To investigate the time-course of hyperglycemia by a single dose	Blood glucose
VI	Epinephrine	Phentolamine (α-adrenergic antagonist)	To investigate the role of α-adrenergic mechanisms in the ability of epinephrine to induce hyperglycemia and hyperlactacidemia	Blood glucose and plasma lactate
VII	Epinephrine	Propranolol (β -adrenergic antagonist)	To investigate the role of β-adrenergic mechanisms in the ability of epinephrine to induce hyperglycemia and hyperlactacidemia	Blood glucose and plasma lactate
VIII	Monocrotophos	Hydrazine sulfate (Gluconeogenesis inhibitor)	To investigate the role of gluconeogenesis in monocrotophos-induced hyperglycemia	Blood glucose and plasma lactate

Monocrotophos (dimethyl (*e*)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) is a fast acting, cholinesterase-inhibiting OP exerting contact and systemic toxicity. In our previous work, we have shown the hyperglycemic and stressogenic outcomes (hypercorticosteronemia and increased liver tyrosine aminotransferase activity) in rats exposed to a single oral dose of monocrotophos and the involvement of AChE-inhibition in the above mentioned effects (Joshi and Rajini, 2012). The present investigation was carried out to obtain further insights into the mechanistic aspects of hyperglycemic and stressogenic impact of monocrotophos in rats. We assessed the influence of glucocorticoid receptor antagonist (mifepristone, RU486), a β-adrenergic receptor antagonist (propranolol) and an α-adrenergic receptor antagonist (phentolamine) on hyperglycemic and stressogenic effects (hypercorticosteronemia and increased liver tyrosine aminotransferase activity) of monocrotophos (1.8 mg/kg b.w., oral, 2 h exposure). The term 'stressogenic' has been used to define the onset of hypercorticosteronemia and induction of liver TAT activity as described earlier (Kassa and Bajgar, 1994). Effect of atropine sulfate (ACh receptor antagonist) was studied on hyperlactacidemia induced by monocrotophos. Further experiments were carried out with exogenous epinephrine with or without pre-treatment with adrenergic antagonists to understand the effect of epinephrine on blood glucose and plasma lactate levels in order to draw commonalities in the action of monocrotophos and epinephrine. The mediation of gluconeogenesis in monocrotophos-induced hyperglycemia was further confirmed by employing hydrazine sulfate, a gluconeogenesis inhibitor.

2. Materials and methods

2.1. Chemicals

Corticosterone, mifepristone (RU486), phentolamine methanesulfonate (PA) and propranolol hydrochloride (PR) were procured from Sigma Chemical Co. (St. Louis, MO, USA). α-Ketoglutarate, bovine serum albumin (BSA), ethylenediaminetetracetic acid (EDTA), l-tyrosine and pyridoxal-5-phosphate were procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). L-Epinephrine hydrochloride and hydrazine sulfate were procured from Loba Chemie (Mumbai, India). Technical grade sample of monocrotophos (76% pure) was a generous gift from Hyderabad Chemical Supplies Ltd. (Hyderabad, India). All other chemicals used in the study were of analytical grade.

2.2. Animals

Adult male rats (CFT - Wistar strain, ~200 ± 5 g) used for the study were housed individually in metallic cages at room temperature (25 ± 2°C) with relative humidity of 50–60% and on a 12 h light–darkness cycle. They had free access to food and water ad libitum. The rats were acclimatized to the commercial diet (Saidurga Feeds and Food, Bangalore, India) for seven days prior to the start of the experiment. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institute Animal Ethical Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. During the experiments, maximum care was taken to minimize animal suffering and in addition, the number of rats used was kept at minimum.

2.3. Animal treatment and experimental protocol

All experiments involving animal sacrifice were conducted in a way that rats were allowed a total fasting period of 24 h at the time of sacrifice. This approach was adopted to study the role of gluconeogenesis for reasons that have been previously discussed (Joshi and Rajini, 2009).

2.3.1. Effect of mifepristone (RU486) on hyperglycemic and stressogenic potential of monocrotophos

Rats were randomly divided into three groups of six animals each. Rats of first group served as vehicle control and received saline containing 25% ethanol (orally) followed by distilled water (orally) 60 min later. Rats of the third group were pre-treated with an oral dose of RU486 (80 mg/kg b.w. in saline containing 25% ethanol) 60 min prior to administration of monocrotophos. The dose of RU486 was based on its protective effects against induction of liver TAT in a preliminary study (data not shown). Rats of second and third groups were orally administered monocrotophos in distilled water (1.8 mg/k.g.b.w.; $1/10LD_{50}$). Rats of all treatment groups were sacrificed 2 h after pesticide administration under mild ether anesthesia. Blood was collected by cardiac puncture into tubes containing EDTA for separation of plasma, which was used for assay of glucose and corticosterone. Liver was excised, cleaned and processed for assay of TAT.

2.3.2. Effect of adrenergic receptor antagonists on hyperglycemia, stress outcomes and hyperlactacidemia induced by monocrotophos

Rats were randomly divided into four groups of six animals each. Rats of first group served as vehicle control and received distilled water (i.p and orally). Rats of third and fourth group were pre-treated intraperitoneally with propranolol (25 mg/kg b.w. in distilled water) and phentolamine (15 mg/kg b.w. in distilled water) respectively 3–5 min prior to administration of monocrotophos. The dose of PA and PR were selected based on their protective effects against hyperglycemic effects of monocrotophos (data not shown). Rats of second, third and fourth group were orally administered monocrotophos in distilled water (1.8 mg/k.g.b.w.; $1/10LD_{50}$). Rats were sacrificed 2 h after pesticide administration under mild ether anesthesia. Blood was collected by cardiac puncture into