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Molybdate partly mimics insulin-promoted metabolic effects in *Drosophila melanogaster*

Bohdana M. Rovenko [a,*], Natalia V. Perkhulyn [a], Oleh V. Lushchak [a], Janet M. Storey [b], Kenneth B. Storey [b], Volodymyr I. Lushchak [a,*]

[a] Department of Biochemistry and Biotechnology, Precarpathian National University named after Vassyl Stefanyk, 57 Shevchenko Str., Ivano-Frankivsk 76025, Ukraine
[b] Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

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ABSTRACT

Molybdenum-containing salts have been found to attenuate diabetes complications in mammals by affecting processes normally regulated by insulin and thus were believed to mimic insulin activity. In this study, we used a fruit fly model to test sodium molybdate, Na_2MoO_4, action in relation to insulin-promoted processes and toxicity. We studied how larval food supplementation with sodium molybdate affected levels of body carbohydrates and lipids in two-day old adult *Drosophila melanogaster*. Molybdate salt, in the concentrations used (0.025, 0.05, 0.5, 5, and 10 mM), showed low toxicity to fly larvae and slightly influenced development and the percentage of pupated animals. Additionally, sodium molybdate decreased the level of hemolymph glucose in males by 30%, and increased the level of hemolymph trehalose in flies of both sexes. These changes were accompanied by an increase in whole body trehalose and glycogen of about 30–90%. Although total lipid levels in flies of both sexes were depleted by 25%, an increased amount of triacylglycerides among total lipids was observed. These effects were not related to changes in food intake. Taken together, the present data let us suggest that sodium molybdate may at least partly mimic insulin-related effects in *Drosophila*.

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1. Introduction

Molybdenum is a cofactor for different enzymes, including xanthine dehydrogenase, sulfite, and aldehyde oxidases and, hence, plays an important role in living organisms (Barceloux, 1999; Mendel, 2013). In several mammalian studies it has been found that administration of molybdate, similar to some other trace transition metals, decreases blood glucose levels by improving glucose utilization within cells, thereby mimicking insulin activity (Shechter and Karlish, 1980; Meyerovitch et al., 1987; Rossetti et al., 1990; Matsumoto, 1994; Ozcelikay et al., 1996; Reul et al., 1997; Yang et al., 2002; Jelikić-Stankov et al., 2007; Vardatsikos et al., 2009; Hua et al., 2012; Vardatsikos et al., 2013). While vanadium and tungsten, trace metals with similar chemical properties, are well studied as insulin mimetics (Shechter and Karlish, 1980; Meyerovitch et al., 1987; Vardatsikos et al., 2009), the ability of molybdenum to regulate blood glucose levels has been poorly investigated. This appears to be related to the rather moderate action of molybdate as insulin mimetic in comparison with that of vanadate, tungstate, and some other trace metals (Fillat et al., 1992; Li et al., 1995a, 1995b; Kierztan et al., 2004; Liu et al., 2004). However, since molybdenum looks more valuable for biological systems than vanadium or tungsten (Mendel, 2013), it might act in different ways than these trace metals. Molybdenum (Mo) is of essential importance for (nearly) all biological systems as it is required by enzymes catalyzing diverse key reactions in the global carbon, sulfur and nitrogen metabolism. One of the possible mechanisms of molybdenum effects includes regulation of molybdenum-dependent enzymes like aldehyde oxidase (Marelja et al., 2014) and xanthine dehydrogenase (Mendel and Bittner, 2006; Mendel, 2013; Schwarz and Belaidi, 2013), which are important for the development of glucose intolerance and diabetic complications (Forbes et al., 2008). The role of molybdenum in the prevention of diabetes and some other age-related diseases might also be mediated by its involvement in the utilization of iron and copper (Mendel, 2013), which are recognized as nutritional risk factors for the development of these pathologies (Brewer, 2010; Khanna et al., 2013; Simcox and McClain, 2013). Recently, Flores et al. (2011) suggested that antagonistic interaction between molybdenum and copper might be involved in the progress of diabetes complications. Therefore, the potential use of molybdenum as insulin mimetic needs further investigation particularly on the potential mechanisms of action.

* Corresponding authors at: Department of Biochemistry and Biotechnology, Vassyl Stefanyk Precarpathian National University, 57 Shevchenko Str., Ivano-Frankivsk 76025, Ukraine. Tel./fax: +380 342 596171.
E-mail addresses: bohdanarovenko@gmail.com (B.M. Rovenko), lushchak@pu.if.ua (V.I. Lushchak).

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In the current study, we investigated the effects of molybdate on the body composition of the fruit fly, *Drosophila melanogaster*. Nowadays, the fruit fly model is extensively used to study diseases associated with impaired carbohydrate and lipid metabolism such as obesity, metabolic syndrome, and diabetes (Baker and Thummel, 2007; Pandey and Nichols, 2011). Exploration of molybdate action on key energetic metabolites in *Drosophila sp.* may provide a perspective approach to disclose the mechanisms involved and revise the role of molybdate in anti-diabetic and obesity prevention therapies.

2. Materials and methods

2.1. Reagents

Potassium phosphate monobasic, sodium phosphate dibasic, sodium chloride, potassium chloride, Coomassie Brilliant Blue G-250, trehalase from porcine kidney and amyloglucosidase from *Aspergillus niger* were purchased from Sigma-Aldrich Corporation (USA). Diagnostic kits for determination of glucose and triacylglycerides were obtained from P.Z. Cormay S.A. (Poland). The kit for total lipid assay was from Erba Lachema s.r.o. (Czech Republic). Sodium molybdate was purchased from Partner Chem. Int. (Poland). All other reagents were of the analytical grade from local suppliers (Ukraine).

2.2. Flies and experimental design

Wild type w^{1118} flies (*D. melanogaster* Meigen) were used in all experiments whereas in a few experiments flies of the Canton-S strain were also used to check if observed effects were strain-related. Stock flies were kindly provided by Bloomington Stock Center (Indiana University, USA). Fly cultures were kept at 25 °C under a 12:12 (light: dark) regime and relative humidity of 55–60%. Parental populations of flies were maintained on yeast-molasses media containing 7.5% (*v*/*v*) molasses, 5% (*w*/*v*) yeast, 6% corn meal (*w*/*v*), 1% (*w*/*v*) agar, and 0.18% (*w*/*v*) methylparaben to inhibit mould growth. For egg collection 300–400 parental flies (3–7 days old) were starved for 2–3 h and then transferred into demographic cages containing a medium with 5% sucrose, 5% yeast, 2% agar, and 0.18% methylparaben. After 6 h, eggs were removed from the medium, washed with distilled water and counted. About 260–280 eggs were put in 250 mL glass flasks with 25 mL of experimental medium containing 5% sucrose, 5% yeast, 1.2% agar–agar, 0.18% methylparaben, and sodium molybdate at different concentrations. Larvae were allowed to develop, pupate, and eclose. Eclosed flies were transferred to fresh food of the same composition including molybdate and held there for two days. Two-day old flies were mildly anesthetized with carbon dioxide gas, separated by sexes, and then quickly frozen in liquid nitrogen for further biochemical analysis.

2.3. Fly development and survival

Survival test. A group of one hundred 48-h old fly larvae reared on the control medium were transferred onto each of the media containing different molybdate concentrations. The percentage (%) of *D. melanogaster* larvae transited into the pupa stage was counted for each group.

Developmental test. The number of pupae formed (%) from larvae grown on control and molybdate supplemented food was counted every 12 h beginning at 86 h after egg laying until the end of the process (180–192 h).

2.4. Assay of food intake

The amount of food consumed was measured using erioglaucine dye (FD&C Blue No. 1, Brilliant Blue FCF) added to the food according to the protocol described in detail previously (Lushchak et al., 2011).

2.5. Preparation of hemolymph and tissue extracts

To collect hemolymph, pre-weighed flies were decapitated and centrifuged (3000 *g*, 6 min, 4 °C) in an Eppendorf 5415R (Germany) centrifuge. Collected hemolymph was diluted in 10 mM phosphate buffered saline (PBS buffer, pH 7.4) in a ratio of 1:5 (mg flies:μL buffer). The diluted hemolymph was heated at 70 °C for 5 min to denature protein followed by cooling to 4 °C. To precipitate denatured proteins, diluted hemolymph was centrifuged (16,000 *g*, 15 min, 4 °C), and resulting preparation was used for measurement of glucose and trehalose levels in hemolymph.

Whole flies were used to obtain body extracts. For this, pre-weighed flies were homogenized in chilled 10 mM PBS buffer (pH 7.4) in a ratio 1:10 (mg flies:μL buffer) at about 4 °C. The protein in resulting body homogenates was denatured and precipitated as described above for hemolymph. Final supernatants were used for assay of body trehalose, glycogen, and triacylglycerides (TAG).

2.6. Glucose, trehalose and glycogen assay

Glucose, trehalose and glycogen levels were measured using a diagnostic kit Liquick Cor-GLUCOSE (PZ Cormay S.A., Poland) following the manufacturer's instructions. Prior overnight incubation of aliquots of diluted hemolymph (10 μL) and body supernatants (10 μL) with trehalase (0.2 I.U.) or amyloglucosidase (0.15 I.U.) at 28 °C was carried out to hydrolyze trehalose and glycogen and then these were measured as glucose units. Standard glucose solutions in a concentration range from 2 to 20 μg/mL were used for determination of glucose levels in all samples. The levels of hemolymph trehalose and body trehalose and glycogen were calculated by subtracting endogenous glucose amounts.

2.7. Triacylglyceride and total lipid assay

Triacylglyceride levels were measured using a diagnostic kit Liquic Cor-TG (PZ Cormay S.A., Poland) following kit guidelines. Standard TAG solutions in the concentration range from 3 to 30 μg/mL were used for determination of TAG content in flies. TAG levels in fly bodies were expressed as micrograms per milligram of fly wet mass (μg/mg wm).

Total lipid levels were measured using a diagnostic kit TOTAL LIPIDS: (TL 180) Erba Lachema s.r.o. (Czech Republic). For this lipids were extracted by homogenization of fly samples in cold 96% ethanol (4 °C) with a Potter-Elvehjem glass homogenizer at a 1:15 ratio (*w*/*v*). Chloroform was then added 1:1 (*v*/*v*), mixed and incubated for 10–15 min, followed by centrifugation (3000 *g*, 2 min, 21 °C) in a CV-1500 centrifuge (USSR). Chloroform fractions (40 μL) were removed and completely evaporated in an air-flow cabinet followed by hydrolysis of the residue by addition of 200 μL of concentrated H_2SO_4 (95%) and incubation at 90 °C for 20 min. After cooling, 200 μL of the reaction mixture was added to each sample. After colour formation for 10 min, 1 mL of concentrated H_2SO_4 was added to each sample. Absorption of samples was determined by spectrophotometer Specoll-211 (Carl Zeiss, Jena, Germany) at 540 nm in a glass cuvette. Standard solutions of total lipids in the concentration range from 16 to 128 μg/mL were used to generate the calibration curve to determine the amount of total lipids in fly bodies. The values of total lipids are expressed as micrograms of total lipids per milligram of fly wet mass (μg/mg wm).

2.8. Total protein assay

Whole flies were homogenized in 50 mM potassium phosphate buffer (pH 7.5) with a Potter-Elvehjem glass homogenizer at 1:10 ratio (*w*/*v*). Homogenates were centrifuged (16,000 *g*, 15 min, 4 °C) in an Eppendorf 5415R centrifuge (Germany). Supernatants were removed and soluble protein concentrations were measured by the

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