Vol. 140, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1986 Pages 51-55

MECHANISM OF MANNOSE TOXICITY

Mónica de la Fuente, Pablo F. Peñas & Alberto Sols*

Instituto de Investigaciones Biomédicas CSIC and
Departamento de Bioquímica, Facultad de Medicina, UAM
Arzobispo Morcillo 4, 28029 Madrid, Spain

Received August 18, 1986

SUMMARY: Mannose toxicity in honeybees is due to a marked shortage of mannosephosphate isomerase that leads to a large accumulation of mannose-6-P and a marked depletion of ATP. Drosophila melanogaster and Ceratitis capitata are insensitive to mannose and have excess of mannosephosphate isomerase over hexokinase. 2-Deoxyglucose is as toxic as mannose for honeybees and is toxic also for the other insects studied, which supports the conclusion that the mechanism of mannose toxicity involves large accumulation of a hexosephosphate.

Mannose has been found to be teratogenic for rat embryos , in vitro (1) and in vivo (2). Mannose was known to be toxic for honeybees because of unbalance between a high hexokinase activity and a low mannosephosphate isomerase activity (3), a conclusion later questioned (4,5) or indirectly supported (6) by others. Now it appeared important to inequivocally ascertain the mechanism of mannose toxicity in bees. The results reported here fully confirm a specific shortage of mannosephosphate isomerase in honeybees leading to a large accumulation of mannose-6-P and a marked depletion of ATP when they ingest mannose.

MATERIALS AND METHODS

Animals: Honeybees (Apis mellifera), Drosophila melanogaster and Ceratitis capitata were obtained from local sources, kindly supplied of Mr. P.F. Grande, Dr. M. Calleja and Dr. M. Muñoz respectively.

Mortality study: Lots of 15 honeybees, 50 Ceratitis and 100 Drosophila were offered 1 M solutions of mannose, glucose or 2-deoxyglucose (Sigma Chemicals Co.). Number of dead animals were counted at time intervals on the basis of lack of any visible movement.

* To whom correspondence should be addressed.

0006-291X/86 $1.50

Enzyme assays. Lots of each of the indicated species weighing 1 to 2 grams were homogeneized with 0.1 M HEPES, 0.1 M KCl, 1 mM $MgCl_2$, 2 mM mercaptoethanol, pH 7.4, in a Polytron homogeneizer; the homogenates were filtered through gauze and centrifuged at 600 x g for 10 min, discarding the sediment. To the supernatants was added Triton X-100 for 1% concentration before assay of the enzymes. Hexokinase was assayed with 0.5 mM mannose and 2 mM ATP, with mannosephosphate isomerase, glucosephosphate isomerase and glucose-6-phosphate dehydrogenase (0.5 U each) by the conventional spectrophotometric method at room temperature with 0.5 mM $NADP^+$ in presence of 0.05 M HEPES, 0.1M KCl, 0.01 M $MgCl_2$, pH 7.4. Mannosephosphate isomerase was assayed with 10 mM mannose-6-P, and glucosephosphate isomerase, glucose-6-phosphate dehydrogenase, $NADP^+$ and the buffer mixture described above. Hexokinase was also assayed with 0.5 mM glucose, 2 mM ATP and glucose-6-phosphate dehydrogenase (0.5 U). Glucosephosphate isomerase activity was determined with 0.5 mM fructose-6-P and glucose-6-phosphate dehydrogenase (0.5 U).

Determination of mannose 6-P and ATP concentrations. Lots of 15 honeybees, were inmersed in dry ice, extracted with 10% perchloric acid, and homogeneized first in a mortar and then in a Polytron homogeneizer, followed by neutralization with 2 M KOH, 0.5 M triethanolamine, and removal of insoluble material by centrifugation. Mannose-6-P and ATP were assayed by conventional enzymatic procedures (7).

RESULTS AND DISCUSSION

The results in Fig. 1 show the marked specificity of the mannose toxicity for honeybees, as illustrated by the contrast be-

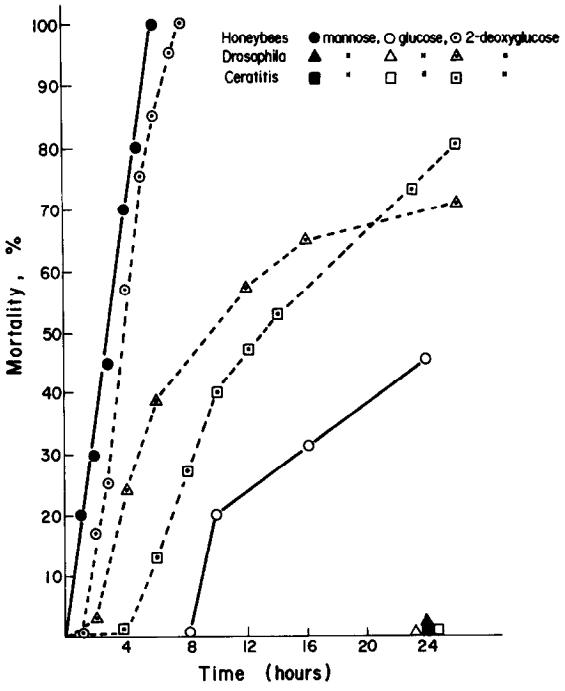

FIG. 1. Mortality induced by mannose in honeybees and by 2-deoxyglucose in honeybees, Drosophila and Ceratitis. 1 M solutions of sugars were offered as described in Materials and Methods.

tween the rapid mortality of bees and the lack of toxicity for two other insects commonly used in laboratory work, namely Drosophila melanogaster and Ceratitis capitata (8), while for all of them is toxic the readily phosphorylable but not further metabolizable 2-deoxyglucose (9). These results also suggest important time differences in the rapid energy metabolism of bees respect to the other insects studied here. Survivals shown in the figure should be related to mortality with water alone, which at 4 and 12 hours were: bees 10 and 100%, Drosophila 15 and 30%, and Ceratitis 0 and 7%.

The main enzymes of mannose metabolism, hexokinase and mannosephosphate isomerase in the three types of insect are shown in Fig 2. Mannose phosphorylation activity is high in all, in the order bees> Ceratitis > Drosophila. Mannosephosphate isomerase is so low in bees that the ratio hexokinase (mannose)/mannosephosphate isomerase is as high as 25, while the other two insects have ample excess of ability to isomerize mannose-6-P over their abilities to phosphorylate mannose. Under similar conditions of assay the phosphorylation of glucose by the homogenates of the three insects was about 1.5 faster than that of mannose, consistent with typical hexokinases, as shown previously for the bees (6,10); and glucosephosphate isomerase activity was 5 to 10-fold that of hexokinase with glucose in all cases.

The time course of the large accumulation of mannose-6-P in bees intoxicated with mannose is shown in Fig. 3, which also shows a simultaneous very marked decrease in ATP levels. In similar conditions glucose-fed bees had 0.05 μmol/g of mannose-6-P and 2.3 μmol/g of ATP.

These results indicate that in honeybees offered mannose a constitutional scarcity of mannosephosphate isomerase in the presence

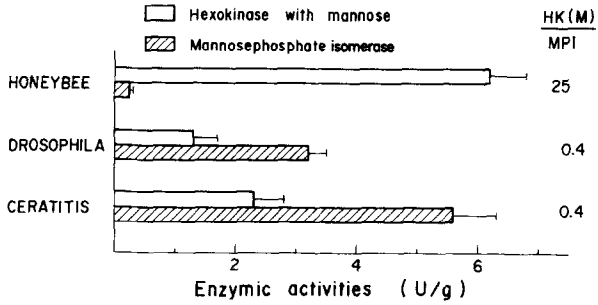

FIG. 2. Hexokinase activity with mannose and mannosephosphate isomerase in honeybees, Drosophila and Ceratitis. Enzymes activities were assayed in homogenates as described in Materials and Methods.

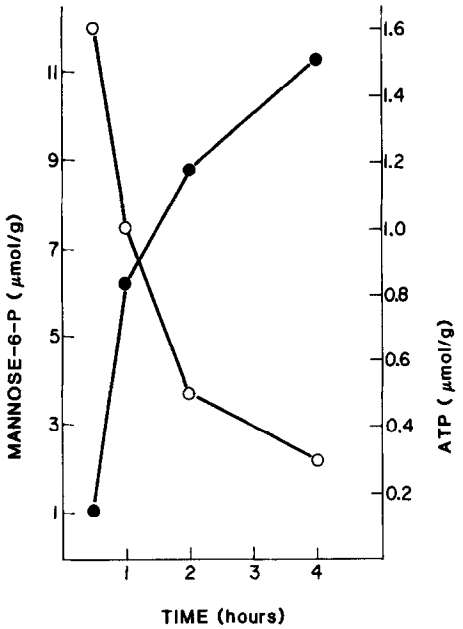

FIG. 3. Accumulation of mannose-6-P (●) and decrease of ATP (o) in honeybees along intoxication by mannose.Lots of 15 bees just taken from the hive were offered 1 M mannose and treated at the indicated times as described in Materials and Methods.

of a high level of hexokinase able to phosphorylate mannose leads to accumulation of high levels of mannose-6-P with a concomitant large decrease of ATP. It is proposed that this mechanism of mannose toxicity in honey bees can serve as a model in the search for the molecular basis of the teratogenicity of mannose for rat embryos. Preliminary results from this laboratory suggest that this mechanism is involved in mannose toxicity for certain tumors (J. Gutierrez, M. de la Fuente and A. Sols).

ACKNOWLEDGEMENTS. This work was supported by a grant from the Fondo de Investigaciones Sanitarias of the Spanish Ministry of Health. We thank E. Lavilla, E. M. López and G. Rábago (medical students) for collaboration in preliminary experiments, and Dr. J. Gutiérrez-Correa for helpful criticism . M. de la Fuente was on leave of absence from the University of Córdoba (Spain).

REFERENCES

1. Freinkel, N., Lewis, N.J., Akazawa, S., Roth, S.I. & Gorman, L. (1984) N. Engl. J. Med. 310, 223-230.
2. Buchanan, T., Freinkel, N., Lewis, N.J., Metzger, B. E. & Akazawa, S.(1985) J. Clin. Invest. 75, 1927-1934.
3. Sols, A., Cadenas, E. & Alvarado, F. (1960) Science 131, 297-298.

4. Saunders, S.A., Gracy, R.W., Schnackerz, K.D. & Noltmann, E.A. (1969) Science 164, 858-859.
5. Handel, E. van (1971)Comp. Biochem. Physiol. 38B, 141-145.
6. Arnold, H., Seitz, V. & Löhr, G.W. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 266-272.
7. Bergmeyer, H.V. ed.(1974) Methods of Enzymatic Analysis. Verlag Chemie, Weinheim .
8. Guillén, A., Haro, A. & Municio, A. M. (1985) Mol. Cell. Biochem. 65, 83-88.
9. Heredia, C.F., De la Fuente, G. & Sols, A. (1984) Biochem. Biophys. Acta 86, 216-223.
10. Ruiz-Amil, M. (1962) J. Ins. Physiol. 8, 258-265.