Research article

Effect of phytohormones on tissue hexokinase and on some blood components in wistar rats

P. Muthuraman1, S. Ravikumar1, J. Vikramathithan1, G. Nirmalkumar1, K. Srikumar*1

*Corresponding author:
K. Srikumar
Dept. of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Kalapet, Puducherry-605014, India.
Email: frenzram@gmail.com

Abstract

Background: Dietary phytohormones may influence the metabolic processes in animal cells. To determine the effect of the plant growth regulators 28-homobrassinolide, gibberellic acid and kinetin on the tissue hexokinase and some blood components in male rats. Methods: 50µg 28-homobrassinolide, gibberellic acid and kinetin (Normal saline served as control) was injected intradermally. Hexokinase activity in several tissues and blood level of glucose, hemoglobin and cholesterol were determined 2 h after injection. Findings: 28-homobrassinolide increased hexokinase activity in all tissues studied, and reduced blood glucose level but increased hemoglobin and cholesterol level. Gibberellic acid increased hexokinase activity in liver, but decreased it in other tissues and increased significantly blood cholesterol and slightly glucose and hemoglobin levels. Kinetin decreased hexokinase activity significantly in liver and kidney, but not significantly in other tissues, and reduced slightly glucose and hemoglobin levels and reduced significantly serum cholesterol level. Conclusion: This study indicated that treatment of rat with 28-homobrassinolide significantly increased hexokinase activity in liver, heart and kidney whereas gibberellic acid increased hexokinase activity only in the liver, and Kinetin decreased hexokinase activity in most tissues. Decreased hexokinase activity was observed due to kinetin and gibberellic acid treatment, except for gibberellic acid in the liver tissue, and was indicative of the fact that kinetin and gibberellic acid acted as negative modulators of this enzyme in the rat tissues. Thus, three different phytohormones possessed different degrees of effect in the animal cells.

Keywords: Hexokinase; Hemoglobin; Gibberellic acid; 28-homobrassinolide; Kinetin.

Introduction

The effect of plant growth regulators (PGRs/phytohormones) on plants is well understood and PGRs are extensively used in agriculture. Knowledge of the effects of phytohormones on animals is however, lacking. Although different phytohormones have been investigated on insects for their specific effects, reports concerning their use on animals remains limited (1, 2, 3). Fecundity, longevity and egg viability are known to be altered in different insects by different phytohormones (4, 5, 6). Cytotoxic effect of kinetin was reported mouse, human and plant tumour cells (7) and Kinetin Provide Ineffective Photoprotection to Skin (8). The regulatory effect of 28-homobrassinolide on glucose
metabolism was reported (9). Ozmen et al (3) observed that abscisic acid and gibberllic acid affected sexual differentiation in mice. PGRs caused increase in the number of splenic plaque forming cells, circulating white blood cells, hematocrit values and thymus weight in young deer mice (10). It is believed that the amount of phytohormones being placed into the environment may exceed that of the use of insecticides (11). Among the phytohormones, a brassinosteroids are a family of recently recognized group of plant growth regulators comprised of polyoxygenated steroid structure. Brassinosteroids exhibit pleiotropic effects as they influenced a variety of developmental processes (12, 13). Gibberellic acid is a member of gibberellins comprising more than ninety molecular species (14). Amongst these the GA$_3$ species is generally used for investigative purposes. Gibberellic acid induced liver neoplasm in Egyptian toads, diagnosed as hepatocellular carcinomas. Gibberllic acid also induced formation of micro abscesses and caused hydropic degeneration in the liver and mononuclear inflammatory infiltration in the rat kidney. Kinetin is a cytokinin, a class of plant hormone that promotes cell division (15). Kinetin has been thoroughly tested for its powerful anti-aging effects in human skin cells and other systems. Hsiao et al., (16) suggested that kinetin was an effective free radical scavenging activity in vitro and an antithrombotic activity in vivo. Kinetin did not affect the lipid peroxidation marker malondialdehyde level in erythrocyte, muscle, heart, kidney and liver tissues. Earlier studies on lipid peroxidation and antioxidant defense by the brassinosteroid isoform 28-homobrassinolide and gibberellic acid indicated both phytohormones acted in an opposing manner in the tissues of normal as well as alloxan induced diabetic male albino rats (17)

The primary aim of this study was to investigate the possible effect of PGRs on the hexokinase enzyme activity in the brain, heart, liver, kidney and testis of male albino rats. The additional objective was to investigate the effect these of PGRs on glucose, 28-homobrassinolide and serum cholesterol level in the same.

Materials and methods
Experiments were carried out in accordance with internationally accepted ethical guidelines for the care of laboratory animals. Male albino wistar strain rats (wt.150 ± 10g) housed in propylene cages under control temperature and hygiene condition with 12 hours of light and dark circle throughout experimental period were used for the investigation. The animals were provided free access to drinking water ad libitum. The technical grade 28-homobrassinolide used in the study was received courtesy of a Dr. Vyas, Godrej Agrovet, Mumbai, India. Gibberellic acid and kinetin were commercially purchased from Himedia, India. Glass distilled water was used for the preparation of all reagents. Four groups of animals were identified and designated, Group I: control rat, Group II: rat treated with 28-homobrassinolide, Group III: rat treated with gibberellic acid, Group IV: rat treated with kinetin. 50 µg of these phytohormones administered through intradermally. The dose level selected based on the some previous experiments. 28-homobrassinolide shows more potential at 50 µg dose levels (18, 26). Hence, we selected same dose level for other two phytohormones for comparison. Control rats were given normal saline.

2 hours post administration of these PGRs, rats were anesthetized with ketamine hydrochloride. Blood was collected from the heart and transferred immediately into disposable silicon coated tubes with EDTA as anticoagulant. The blood samples were used for determination of glucose, hemoglobin and cholesterol. Tissues (brain, heart, liver, kidney and testis) were removed surgically and tissue homogenates (10% w/v) were prepared in 1.15% Kcl adjusted to pH7.5 for the determination of hexokinase enzyme activity.

Tissue hexokinase/glucokinase activity was estimated using a coupled enzyme assay method (18). Blood glucose level was estimated by Asatoor and King Method (19). Serum cholesterol was estimated by Zak’s method (20). Hemoglobin content of blood was estimated by standard method (21).

Statistical analysis
The experimental data was analyzed using the ANOVA. Differences were considered significant when p< 0.05.

Result
Administration of 28-homobrassinolide, gibberellic acid and kinetin caused changes in tissue hexokinase enzyme activity, blood glucose, hemoglobin and
cholesterol contents in male albino rats.

Table 1. The effect of plant growth regulators on hexokinase enzyme activity in brain, heart, liver, kidney and testis of male albino rats and expressed as IU x 10^{-2}/ml.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brain</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19 ± 1</td>
<td>14 ± 0.7</td>
<td>26 ± 1.5</td>
<td>21 ± 1.7</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>28-homobrassinolide treated</td>
<td>27 ± 1.6*</td>
<td>23 ± 1.2** ↑64.3%</td>
<td>37 ± 1.5** ↑42.3%</td>
<td>29 ± 1* ↑38%</td>
<td>19 ± 0.8 ↑18.7%</td>
</tr>
<tr>
<td>Gibberellic acid treated</td>
<td>17.7 ± 1</td>
<td>12 ± 0.4 ↓14.3%</td>
<td>33 ± 2* ↑26.9%</td>
<td>15.5 ± 1 ↓26.2%</td>
<td>15 ± 0.5 ↓6.2</td>
</tr>
<tr>
<td>Kinetin treated</td>
<td>16 ± 1</td>
<td>13 ± 0.8 ↓7.1</td>
<td>19 ± 0.5* ↓26.9</td>
<td>16 ± 0.6 ↓23.8</td>
<td>17 ± 0.7 ↑5.9</td>
</tr>
</tbody>
</table>

\( n = 6 \) rats; \( n = 3 \) replicates; *\( P < 0.05 \) (significant), **\( P < 0.01 \) (highly significant); ***\( P < 0.001 \) (extremely significant).

Hexokinase enzyme activity was significantly elevated 42.1 and 64.3 \% in brain and heart respectively whereas 42.3 and 38\% in liver and kidney by 28-homobrassinolide. 28-homobrassinolide moderately increased hexokinase activity in the testis (18.7\%) (Table 1). The blood glucose was down regulated upto 19\% from control value where as hemoglobin increased 17.9\% over the control by 28-homobrassinolide. 28-homobrassinolide increased the serum cholesterol level to 12.9\% (Table 2). Hexokinase enzyme activity was significantly increased 26.9\% in liver, whereas in other tissues the hexokinase activity was decreased by gibberellic acid treatment (Table 1). The blood glucose and hemoglobin levels were slightly elevated but serum cholesterol level increased very significantly by gibberellic acid (24\%) (Table 2).

Table 2. The effect of plant growth regulators on glucose, hemoglobin and cholesterol content in male albino rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>28-homobrassinolide treated</th>
<th>Gibberellic acid treated</th>
<th>Kinetin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>84.0 ± 4</td>
<td>68.0 ± 3.2*** ↓19%</td>
<td>89.0 ± 3.1* ↑6%</td>
<td>82.7 ± 3 ↓1.5%</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>9.5 ± 0.4</td>
<td>11.2 ± 0.7* ↑17.9%</td>
<td>9.6 ± 0.6 ↑1%</td>
<td>9.3 ± 0.3 ↓2.1%</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>42.5 ± 3</td>
<td>48.0 ± 2.7* ↑12.9%</td>
<td>52.7 ± 2.6 ↑24%</td>
<td>30.2 ± 1** ↓29%</td>
</tr>
</tbody>
</table>

\( n = 6 \) rats; \( n = 3 \) replicates; *\( P < 0.05 \) (significant), **\( P < 0.01 \) (highly significant); ***\( P < 0.001 \) (extremely significant).
Hexokinase enzyme activity was significantly decreased in liver and kidney whereas not significantly reduced in brain, heart and testis by kinetin administration (Table 1). The blood glucose and hemoglobin content were slightly reduced whereas serum cholesterol content significantly was reduced 29% in the rats by kinetin (Table 2).

**Discussion**

In this investigation, the phytohormones 28-homobrassinolide, gibberellic acid and Kinetin were selected since information on their positive or negative effect on rat tissues in vivo remains limited. The data collected was for a single time point study. We reported earlier an antidiabetic potency of 28-homobrassinolide (9). Dietary and mobilized glucose being utilized by cellular glycolysis, hexokinase acted as a first step in the metabolic sequence as a phosphorylating enzyme. Hence, the rate of glycolysis (glucose utilization) mainly depended upon the level of hexokinase activity in a cell (22). We therefore focused on this enzyme activity. This study indicated that treatment of rat with 28-homobrassinolide enhanced hexokinase activity in liver, heart and kidney whereas gibberellic acid enhanced hexokinase activity only in the liver, and Kinetin decreased hexokinase activity in most tissues. Indole acetic acid, another phytohormone, does not affect hexokinase enzyme activity significantly when administered intraperitonially (23). Oral administration of gibberellic acid for 7 consecutive days increased malondialdehyde and 4-hydroxyl-2-nonenal levels in rat tissues and erythrocytes whereas 28-homobrassinolide revealed an opposite effect (24). This work indirectly indicated the antidiabetic potency of 28-homobrassinolide (Hexokinase plays major role in glucose utilization by phosphorylating glucose). Reduced glutathione content and catalase activity was increased by 28-homobrassinolide and reduced by gibberellic acid when these compounds were administered intradermally at acute level (24). The antidiabetic potency of 28-homobrassinolide remained upto 144 hours when 28-homobrassinolide was given orally as established by our earlier investigation (9). Our work is closely related to the previously published article (25). 28-homobrassinolide increased HK I mRNA expression in normal rats (26). The biological and metabolic consequences of low dose homobrassinolide used in rats, seem to indicate enhancement of glucose phosphorylation leading to greater glucose entry into the cells of each tissue and utilization of the G-6-P generated by hexokinase either through glycolysis or through the HMP pathway. Since the utilization of glucose was monitored by the coupled enzyme assay employing NADP as cofactor, a concomitant activity increase in G-6-PD was noted that ensured the utilization of G-6-P through the HMP pathway, whenever greater amounts of G-6-P was generated within the cells. It therefore follows that generation of NADP and ribose will be augmented in the cells due to the homobrassinolide effect. The decrease in blood glucose content by 19% and increase in hemoglobin content by 28% were both indicative of the fact that homobrassinolide was a potent bioregulator especially of circulating glucose level in the rat. 28-homobrassinolide increased hemoglobin and cholesterol content while Kinetin reduced the cholesterol content.

Decreased hexokinase activity was observed due to kinetin and gibberellic acid treatment, except for gibberellic acid in the liver tissue, and was indicative of the fact that kinetin and gibberellic acid acted as negative modulators of this enzyme in the rat tissues. Thus, three different phytohormones possessed different degrees of effect in the animal cells. Further work is going on to define the molecular mechanism of action of these phytohormones in rat cells.

**Conclusion**

This study indicated that treatment of rat with 28-homobrassinolide significantly increased hexokinase activity in liver, heart and kidney whereas gibberellic acid increased hexokinase activity only in the liver, and Kinetin decreased hexokinase activity in most tissues. Decreased hexokinase activity was observed due to kinetin and gibberellic acid treatment, except for gibberellic acid in the liver tissue, and was indicative of the fact that kinetin and gibberellic acid acted as negative modulators of this enzyme in the rat tissues. Thus, three different phytohormones possessed different degrees of effect in the animal cells.

**Acknowledgement**
The authors gratefully acknowledge the financial support received as grants from the Department of Science and Technology (SR/SO/AS-16/2004), New Delhi, India.

References

11. Mickel LG. Plant Growth Regulators controlling biological behavior with chemicals. Chemical & Engineering News 1978; 56:18