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Simulated Hypogravity: Effect on Glutamic Acid Metabolism in Tagetes Patula

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Abstract

L. Proline-UL-^{14}C was fed to shoots of intact 51 day old Tagetes patula plants grown normally or on a horizontal clinostat rotating at 15 rev/hr. After 24 hr. incubation, ^{14}C incorporated into different amino acids of different tissues (stem plus leaves, and flowers) was determined by autoradiography. Also glutamic acid decarboxylase activity in the leaves of control and clinostated plants was determined. It was observed that glutamic acid decarboxylase activity in the control plants was about 2-fold that in gravity compensated plants (grown on horizontal clinostats). In the floral shoot of control plants, about 62% of the total radioactivity, which was incorporated into the amino acid fraction, was lodged in aminobutyric acid (GABA). In contrast, GABA was not labelled in the gravity compensated plants. Rather, practically all the radioactivity (100%) was incorporated into what was suspected to be a peptide.

The interpretation of these results is that under normal conditions the enzyme glutamic acid decarboxylase is active and so glutamic acid is converted to aminobutyric acid by decarboxylation reaction. Under hypogravity, this enzyme is perhaps inactive and another enzyme probably a glutamyl transferase system becomes operative and utilises the glutamate for peptide formation. A scheme showing the biogenic relationship between proline, glutamic acid, aminobutyric acid and peptides is discussed.

Introduction

In a previous report (Oputa and Mazelis 1974) it was shown that hypogravity markedly depressed CO_2 output in Tagetes patula. In this same study an indirect evidence was given that glutamate decarboxylase system might be sensitive to hypogravity.
The present study seeks to test the direct effect of hypogravity on glutamic acid decarboxylase.

Materials and Methods

Tagetes patual var Helen Chapman A was used. The growth condition in the green house were described in a previous report (Oputa and Mazelis 1976).

Preliminary Study on Glutamate Metabolism Incubation Procedure

An aliquot of L-Proline - UL - 14^c solution containing 20 UCL was fed to the stems of two 51 day old potted 4 rph (hypogravity) the other left on the bench as control. A modified cotton wick method involving the use of capillary tubing (Oputa and mazelis 1976) was employed in the label feeding. The plants were under fluorescent light with an intensity of 40 ueinsteins/ m^2/sec in the spectrum between 400 and 700 um. After 24 hours incubation, the shoots were cut just above the point of label feeding, and separated into stem plus leaves, and flowers. These tissues were extracted separately as described below.

Extraction Procedure

The tissues were transferred into 50ml of boiling 80% ethanol for 3 minutes. The ethanolic extract was decanted and the tissue ground with mortar and pestle. The ground tissue and ethanolic extract were combined in a 125 ml Erlenmyer and shaken for thirty minutes in a reciprocating shaker. The suspension was centrifuged and the residue was successively extracted with 80% ethanol, water and 80% ethanol. The extracts were combined and filtered through celite mat. The volume of the extract was measured and an aliquot was assayed for radioactivity by means of a Nuclear chicago scintilation counter. The extract was taken to dryness under reduced pressure at 30°C.

Column Chromatography:

The dried extract was taken up in 80% ethanol and an aliquot was put on a 1 x 10cm column of Dowex 50 W x 4 (H^+). The column was eluted with 80% ethanol to remove sugar and organic acids. The column was next eluted with ethanolic aqueous ammonia (3 parts 75% ethanol + 1 part concentrated ammonium hydroxide). This fraction was the amino acid fraction. An aliquot of this fraction was assayed for radioactivity. Radioactivity assay for sugars and organic acids were also carried out on anion exchanger (Dowex 1 x 8 (HCOO-). For the purpose of this purpose of this paper, however, only results for the amino acid fraction are reported.

Paper Chromatography of amino acid fraction:

The components of the amino acid fraction were separated by two dimensional chromatography on Whatman No 1 filter paper. The solvent system employed was phenol: water (10:2 v/v) and butanol; acetic acid: water (12:3:5 v/v). Radioautograph of the paper chromatogram was made on kodak No 1 screen medical X-ray film.

Radioative areas on the chromatogram were located by matching the paper with the X-ray film. The radioactive areas were cut and the papers rolled into cylinders and immersed in Bray's scintillater. Non-radioactive area near the origin was used as blank. Radioactivity was assayed by means of Nuclear Chicago scintillation counter. Amino acid spots on duplicate chromatograms were located with ninhydrin spray.

Glutamate decraboxylase Experiment:

A 60 day-old plant was mounted on a horizontal clinostat (hypogravity) while a similar plant was left on the bench as control. Forty eight hours after epinastic curvatures were evident on the clinostated plant, two opposite leaves just below the floral buds were harvested for glutamic acid decarboxylase assay. Similar leaves on the control plant were also harvested.

Preparation of Crude Enzyme:

0.8g leaves of each treatment were ground with 0.1 M K_2PO_4 buffer PH 7.2 in a precooled mortar and pestle. The suspensions were filtered through four layers of cheese cloth and centrifuged (3000 rpm x 5 min). The supernatant was decanted and recentrifuged. The supernatant was made up to 8.0 ml and aliguots of each were boiled. This boiled extract is designated "boiled enzyme". All enzyme preparations were kept in an ice bucket.

Radioactive Material:

L-Glutamic acid-UL-^{14}C with specific activity of 175 mc/mM was supplied by Biochem Nuclear Corporation, Burbank, U.S.A.

Enzyme Assay

The assay mixture consisted of 0.9 ml unlabelled .05 M L-Glutamic acid, 0.625 uCi of L-Glutamic acid-UL-^{14}C in. .05 ml, and 00.5 ml of either crude enzyme or boiled enzyme in a total reaction volume of 1.45 ml. A carbon dioxide trap device involving the use of 0.2 ml Hyamine in a center "well" as described by Fox (1971) was employed. The reaction was started by addition of the enzyme preparation. Incubation period was for 15 minutes, the vessels being shaken by means of a wrist arm shaker. The reaction was stopped by addition of 0.2 ml 10% T.C.A. The vessels were shaken for another 30 minutes to ensure complete carbon dioxide trap. The vial with hyamine was wiped dry and transferred into a scintillation vessel containing 15.0 ml Bray's scintillation fluid. The mixture was thoroughly mixed and counted by means of a Nuclear Chicago Scintillator.

Results

Data on label incorporation (table 1) indicate that vegetative and floral tissues respond differently to sero gravity (Og):

The amount of label incorporated into the vegetative tissues was not affected much by Og. In contast, the floral shoot showed a marked response to Og especially in terms of the amount label that was lodged in the amino acid fractions. Thus while only 17.5% of total radio-activity was found in the amino acid fraction from flowers of control plants, as much as 47.2% of total radio-activity was detected in the same fraction from flowers of plants subjected to Og.

Autoradiography of the amino acid fractions from control and Og flowers further revealed staggering differences in label distribution amongst the constituent amino acid. (Table 2): In the flowers of control plants, -amino butyric acid (GABA) contained the greatest percentage of label (62.1%) while only small amounts of label (3.4 - 11.1%) were found in, aspartate, glutamate and glutamine. In the flowers of Og plants, however, GABA was not labelled at all. Instead, one of two spots suspected to be peptides (because of their location on the chromatogram) accumulated practically alll the radioactivity (— 100%). Only in-significant trace amounts of label were detected in aspartate and glutamate: Figure 1 indicates clearly that glutamic acid decarboxylase activity in leaves subtending the floral shoot of plants under Og was decreased by 50% when compared with the activity of this enzyme in similar tissue of control plants.

TABLE 1: LABEL INCORPORATION INTO AMINO ACID FRACTION IN TISSUES OF CONTROL PLANTS AND HYPOGRAVITY PLANTS AFTER 24 HR INCUBATION WITH L-PROLINE -UL- ^{14}C

RADIOACTIVITY: CPM(GFR Wt)-1.

(Stem + Leaves) Control	(Flowers) Hypogravity	Control	Hypogravity	Total ^{14}c
Total ^{14}C incorporated	4.54×10^6	4.24×10^6	1.63×10^6	1.40×10^5
Amino acids	6.68×10^5	4.52×10^5	2.85×10^5	6.60×10^5
	(14.7)	(10.7)	(17.5)	(47.2)

Figures in bracket represent % of total ^{14}C incorporated

TABLE 2: DISTRIBUTION OF ^{14}C AMONGST AMINO ACIDS IN THE FLORAL SHOOT OF T. PATULA VAR HELEN CHAPMAN A AFTER 24 HR INCUBATION WITH L-PROLINE-UL- ^{14}C

Amino Acids	**% Distribution of Label. Control**	**Hypogravity**
Aspartic acid	3.4	**Hypogravity**
Glutamic acid	11.1	**Trace**
Glutamince	10.1	Nil
— Amino butyric acid	62.1	Nil
Basic amino acids (unidentified)	8.3	Nil
Others?	4.5	Nil" Peptide"*
	—	100
Peptide**	—	Trace

* Percentages based on total activity recovered from the paper chromatogram.

** Not identified as such but suspected to be peptides.

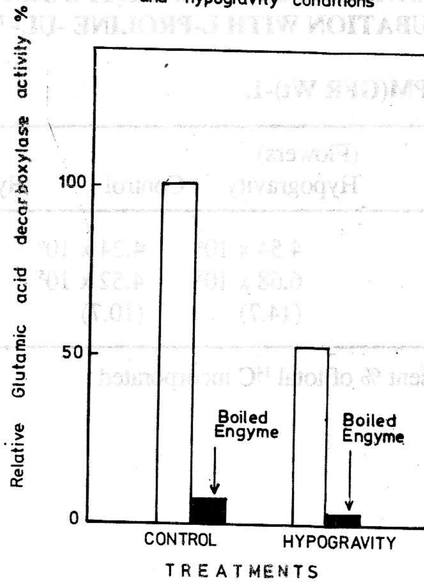

Fig. 1

Relative glutamic acid decarboxylase activity ion leaves subtending flowers of T. patula plants grown under normal (control) and hypogravity conditions.

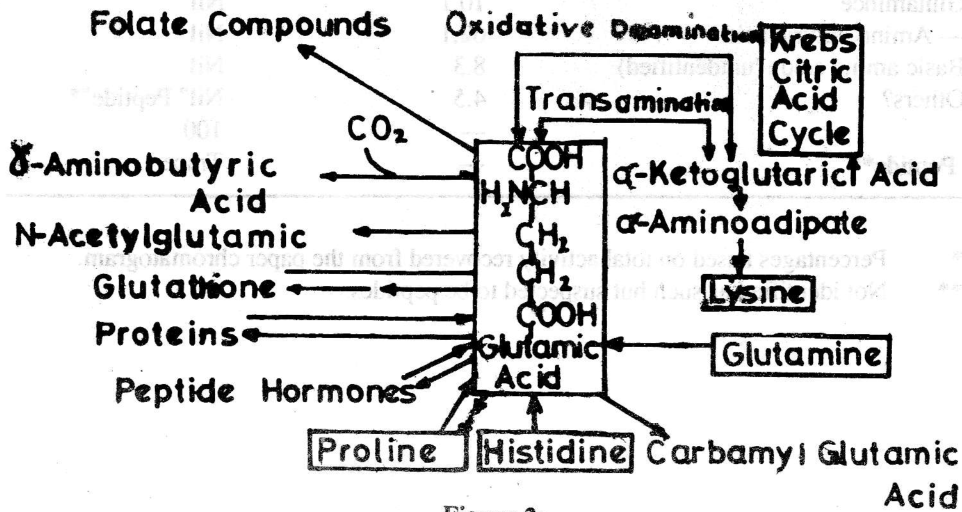

Figure 2:

Biogenic Interrelationships between proline, glutamic acid, aminobutyric acid and peptides. (After Bennett 1968).

Discussion

The absence of -amino butyric acid (GABA) in clinostated plants suggests a zero gravity induced impairment of glutamic acid metabolism. That this was indeed the case is evidenced by the marked decrease in glutamic acid decarboxylase activity observed in this study. Under normal conditions, glutamic acid is decarboxylased by this enzyme to produce GABA. Inactivation of the enzyme should be accompanied by the accumulation of glutamic acid assuming that this amino acid is not broken down. Results from autoradiography shows very clearly that only trace amounts of radio-activity was detectable in glutamate from Og plants — an evidence that glutamic acid did not accumulate. Apparently, the glutamate was chanelled towards the synthesis of another compound, probably the compound that incorporated practically all the radioactivity in the amino acid fraction. The chemical identity of this compound was not established in this study. However, from its position on the chromatogram it was suspected to be a peptide. It appears that when glutamic acid decarboxylase is inactivated by Og perturbation, another enzyme, probably a glutamyl transferase system, becomes operative and utilises the glutamate for peptide formation. A ready flow of labelled carbon from the fed substrate, Proline UL-14C, to GABA and peptides is possible considering the intimate biogenic relationship between proline and these compounds. Figure 2 outlines these relationships (Bennett 1968).

The sensitivity of the glutamic acid decarboxylase system of T. patula to weightlessness enlists it as a good physiological marker for studies in outer-space environments. There is also the possibility that some peptide synthesizing system is equally sensitive. It would be worthwhile to establish whether these metabolic indicators are applicable to other plants or other bio-systems. Also an important question arises from the results of this study: What is the physiological implication of the hypogravity induced disturbance of glutamic acid metabolish?

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