Effect of exogenous supply of amino acids, amide, urea and ureide on free NH_4^+ level in mung beans[1]

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Summary. The results of experiments on the possible source(s) of ammonia for the mung bean are reported. Different nitrogenous metabolites were fed to primary leaves of mung bean seedlings with and without methionine sulphoximine (MSO), an inhibitor of glutamine synthetase (GS). Their role in foliar ammonia loss is discussed.

Ammonia is formed in higher plant cells as a product of the primary pathway of nitrate assimilation[2], during photorespiration[3] and the catabolism of various aminoacids[4] and translocatory compounds like amides and ureides[5]. Further, ammonia may also be present as a result of its direct absorption[6]. Metabolism of NH_4^+ is of importance because its accumulation may affect photophosphorylation and disrupt chloroplast ultrastructure[7,8]. Further, during the recent past, a few laboratories have reported nitrogen loss, predominantly in the form of ammonia, from the foliage of different crop and weed species, particularly under stress and high temperatures[9,10]. This laboratory is interested in elucidating the roles of various metabolic pathways involved in the production, accumulation and subsequent loss of ammonia from the foliage into the atmosphere.

In this communication, effects of various nitrogenous metabolites; glycine, asparagine, cystine, arginine, urea, allantoic acid and phenylalanine on the endogenous levels of free NH_4^+ are described, and their significance in relation to NH_3 loss is discussed.

Materials and methods. The primary leaves of 14-day-old, pot grown mung bean (*Vigna radiata* L. Wilczek) seedlings were used for the study. The seedlings were cut 5 cm below the primary node under water, placed in different treatment solutions and exposed to a light source (300 μE m^{-2} sec^{-1}) for 4 h. The concentrations of the various N compounds tested were similar to those used by other investigators and are comparable to in situ concentrations[11-14]. Subsequently, the leaves were cut into slices (2–4 mm) and homogenized in chilled potassium phosphate (25 mM) buffer pH 7.2 containing 1% polyvinylpyrrolidone (PVP), using a pestle and mortar. The homogenate was centrifuged at 8000 × g for 15 min at 4 °C. Ammonia was transferred by diffusion from buffered (pH 11) aliquots of extracts into 2% boric acid in closed Warburg flasks.

The amount of ammonia was determined using the procedure of Novozamsky et al.[15], with standards prepared in boric acid solution. L-Methionine-DL-sulphoximine (MSO), isonicotinylhydrazide (INH) and allantoic acid were procured from Sigma Chemicals, USA.

Endogenous NH_4^+ levels in the primary leaves of mung bean as affected by exogenous source of different nitrogenous metabolites

Treatment	NH_4^+ μmoles g fresh wt^{-1} h^{-1}	% Change
Experiment No. 1		
a Control	2.71	100
b MSO	6.60	243
c INH	2.65	98
d Glycine	1.91	71
e Glycine + MSO	9.03	333
f Glycine + INH	2.45	90
g Glycine + MSO + INH	6.30	232
Experiment No. 2		
a Control	1.92	100
b MSO	4.45	232
c Asparagine	2.46	128
d Asparagine + MSO	5.92	308
e Cystine	1.70	89
f Cystine + MSO	3.75	195
Experiment No. 3		
a Control	1.91	100
b MSO	5.03	262
c Arginine	2.07	108
d Arginine + MSO	4.29	223
e Urea	2.17	113
f Urea + MSO	5.28	275
Experiment No. 4		
a Control	1.22	100
b MSO	3.16	259
c Allantoic acid	1.56	128
d Allantoic acid + MSO	4.07	333
e Phenylalanine	1.02	84
f Phenylalanine + MSO	3.31	271

MSO, 4 mM; INH, 25 mM; glycine, 15 mM; all other metabolites, 10 mM.

Results and discussion. The level of free ammonia in the control tissue varied from 1.22 to 2.71 μmoles in the 4 experiments. Although the age of the tissue was the same, the variation in the endogenous level may be due to the fact that the 4 experiments were conducted on different days. With addition of MSO there was an accumulation of 2.43 μmoles NH_4^+ g^{-1} fresh wt h^{-1}, whereas INH alone did not have any effect. However, on addition of glycine along with MSO there was substantial increase in NH_4^+ level (see table experiment 1). Treatment (g) showed that this increase was inhibited by INH, thereby providing evidence that increase in the ammonia level was due to photorespiratory ammonia production[11]. Upon feeding asparagine without MSO there was only a slight increase in NH_4^+ level compared to the control but in the presence of MSO, asparagine increased the free NH_4^+ level substantially over MSO alone (see table, experiment 2). This increase in ammonia may result from asparagine degradation by asparaginase[5] or by aminotransferase linked deamidation[13]. Treatment with cystine did not enhance NH_4^+ level.

Experiment 3 shows that neither arginine nor urea, which in situ could be derived from arginine through the ornithine cycle[14] had any influence on NH_4^+ level.

Allantoic acid feeding increased the NH_4^+ level slightly. Addition of MSO with allantoic acid showed an increase of 1.0 μmole NH_4^+ g^{-1} fresh wt h^{-1}, as compared to MSO alone. This suggests a possible role for allantoic acid as a source of NH_4^+. It is known that in some legume species this is the major form of N translocated. Phenylalanine feeding did not show any influence on NH_4^+ level (see table, experiment 4).

Our results show that among the different nitrogenous compounds tested for ammonia production, only glycine, asparagine and allantoate are found to release considerable amounts of free ammonium in the cotyledonary leaves of the mung bean. Since the concentrations of the N metabolites tested were similar to those found in situ, it is likely that these compounds serve as possible sources of ammonia production and its subsequent utilization or loss. The predominance of a particular metabolite and/or pathway may vary with species, age of tissue etc. It is known that in higher plant leaves assimilation of ammonia occurs via an efficient glutamate synthase cycle, though 2 other, less

efficient enzyme systems – glutamate dehydrogenase and asparagine synthetase – are also present[11,16,17]. In spite of the presence of these enzymes, substantial quantities of ammonia are reported to escape from the foliage into the atmosphere[10]. It is possible that limitations of acceptor molecules and/or energy may be the factors responsible for the accumulation of ammonia and its release from the foliage, and the process may be considered as a part of the ammonia detoxification mechanism.

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Effect of prolonged inhibition of histidine decarboxylase on tissue histamine concentrations[1]

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Summary. In rats, chronic infusion of α-fluoromethyl histidine, a selective irreversible inhibitor of mammalian histidine decarboxylase, caused a marked depletion of histamine in all tissues examined. There were no gross pharmacological effects associated with this depletion.

Histamine is formed by decarboxylation of histidine by the specific histidine decarboxylase (E.C. 4.1.1.22)[3] and is present at varying concentration in almost all mammalian tissues[4]. One of the possible approaches to studying the role of histamine in mammalian physiology is by blocking its synthesis using specific inhibitors of histidine decarboxylase. We report here that α-fluoromethyl histidine, a selective enzyme-activated inhibitor of histidine decarboxylase[5–8], given to rats by infusion over 3 weeks, decreases the concentration of histamine in all tissues examined.

Materials and methods. Materials. α-Fluoromethyl histidine was synthesized in our laboratory by a method to be published elsewhere. Histamine, histidine and S-adenosyl methionine were purchased from Merck, Darmstadt, FRG. L-[1-^{14}C]histidine (50 mCi/mmole) came from New England Nuclear Corporation, Boston, USA. L-[2,5-^{3}H]histidine (40–50 Ci/mmole) and S-adenosyl-L-[methyl-^{3}H]-methionine (80 Ci/mmole) were supplied by the Radiochemical Center, Amersham, England.

Animals and treatment. Male rats of the Sprague-Dawley strain were used in this experiment. For chronic infusion of α-fluoromethyl histidine, Alzet 2001 minipumps loaded with 100 mg of coumpound in 200 µl of water were implanted in the peritoneal cavity under light ether anesthesia. The minipumps delivered a flow rate of 1 µl/h for 1 week. Every 7 days the minipumps were renewed. Control animals were sham operated but no minipumps were implanted. The animals were weighed daily and rectal temperature (Appelab thermosonde) was measured at intervals. Gastric secretion was measured on given days by a technique which consisted of washing the stomach with 2 ml of warm physiological saline via a gastric sonde[9]. The pH of the recovered solution was recorded. The animals were killed on day 21.

Tissue preparations. At the end of the chronic treatment rats were killed by decapitation and different organs were removed. All the tissues used were homogenized in 5 vols of phosphate buffer (2×10^{-3} M, pH 7.9 containing 0.1% Triton X100). The crude homogenates were used for determination of histidine decarboxylase activity where appropriate. The supernatants obtained after centrifugation were used for measurement of histamine.

Analysis. Tissue histamine content was determined by a modification of the enzymatic isotopic assay described by Taylor and Snyder[10]. Histidine decarboxylase activity in gastric mucosa was determined by the measurement of $^{14}CO_2$ libeerated from L-[1-^{14}C]histidine. Both methods have been described in detail in a previous publication[8].

Hypothalamus histidine decarboxylase activity was measured by a modification of the radiochromatographic procedure described by Baudry et al[11]. The incubation mixture (70 µl) consisted of 50 µl of tissue homogenate, 1 µCi of purified L-[2,5-^{3}H]histidine (about 2×10^{-7} M), 10^{-5} M of pyridoxal phosphate, 10^{-4} M of histamine and 5×10^{-2} M of phosphate buffer pH 7. After 1 h incubation at 37 °C, the enzymatic reaction was stopped by addition of 10 µl of 2.4 M perchloric acid. After addition of 600 µl of 0.2 M Tris-HCl buffer pH 8, the [^{3}H]histamine formed was isolated by ion-exchange chromatography on Amberlite CG 50 (200–400 mesh).

Results and discussion. In mice, α-fluoromethyl histidine given acutely decreased the concentration of histamine in