The results (Table II) indicate that if T-poly remained in contact with host plants for two days, virus multiplication was reduced in test plants to the extent of 70%. However, the virus inhibitory activity of T-poly declined with time. On the 12th day of virus challenge, assay indicated that the concentration of virus in treated and control plants were about the same.

Discussion

Our investigations show that pre-inoculation treatment of nonhypersensitive *N. tabacum* CV. NP–31 plants by rubbing with a single dose (2·5 mg/ml) of T-poly retarded TMV multiplication in such host plants, as judged by local lesion assay, down to 70% of the control figure (Table II) on the 4th day of virus inoculation. Acquired antiviral resistance induced locally at treated site (basal leaf) spread to the remotest part (upper leaves) as well. Both effects weared off with time ; the concentration of virus (local lesion forming units), as high as in the untreated control, was recovered from treated plants on the 12th day (Table II). Results obtained with the nonhypersensitive host plants here (NP-31) are in agreement with those of VanLoon[11] who reported that *N. tabacum* (Samsun) plants, like its hypersensitive counterpart, have the capacity to elaborate new soluble leaf proteins upon infection with TMV, the appearance of which is associated with induced acquired systemic resistance.

Results of tests showed that resistance to virus in treated *N. tabacum* CV. NP-31 plants develop systemically at remote site (upper untreated leaves) in parallel with virus inhibited locally at the site of treatment (basal leaves). Both local (treated site) as well as systemic resistance (untreated site) induced by a single dose pretreatment were transient in nature.

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ON THE OCCURRENCE OF ARGINASE IN THE MUSCLE AND ITS ROLE IN VARIOUS TISSUES OF FROG, *RANA HEXADACTYLA*, DURING DENERVATION ATROPHY AND CHRONIC AMMONIA TOXICITY

C. SREERAMULU CHETTY, R. CHANDRAMOHAN NAIDU*, W. RAJENDRA AND K. S. SWAMI*
Department of Zoology, S.V. University, Tirupati 517 502

Abstract

Arginse activity was increased in the gastrocnemius muscle, brain, liver and kidney tissues following unilateral sciatectomy and induced ammonia intoxication. The increase in enzyme activity was more pronounced in brain and muscle tissues of both the normal and denervated frogs as compared to liver and kidney during induced ammonia stress. The similarity in the pattern of arginase response to surgical denervation and imposed ammonia toxicity has been discussed.

Introduction

It is well documented that sciatectomy results in the elevation of ammonia levels in various tissues of frog[1–3]. Animal tissues are extremely sensitive even to low concentration of ammonia in their environment, since it is highly toxic[4]. Hence, an efficient means of nitrogen disposal will be required in order to keep the ammonia content at a low level. The major mechanisms involved in the detoxification of ammonia constitute the synthesis of glutamine and urea[5]. Very scant attention has been paid to the ammonia detoxification mechanisms in denervation atrophy. Earlier report have shown that the administration of ammonium salts into animals result in the elevation of tissue ammonia levels which warrants rapid detoxification[2,6]. Since

* Present address : Department of Zoology, Govt. College, Anantapur,

such studies on a comparative basis have not been made earlier, involving denervation atrophy and induced chronic ammonia stress, the present study aims to fill this lacuna. Arginase is one of the enzymes which plays a key role in the urea cycle[7]. A study of arginase activity will throw much light on the possible detoxification of enhanced ammonia in denervation atrophy and ammonia stress.

Materials and Methods

Batches of frogs were unilaterally denervated under aseptic conditions[8]. The muscle with intact innervation was designated as the contralateral muscle (cm), while the neurectomised muscle was designated as the denervated muscle (dm) and the muscle of normal frog was designated as nm. After due standardization, three selected doses of ammonium lactate was administered intraperitoneally through 0·5 ml of physiological saline and the doses were designated as

Mild dose = 1·5 mg of ammonia/kg body weight
Moderate dose = 2·5 mg of ammonia/kg body weight
Sublethal dose = 5·0 mg of ammonia/kg body weight

Ammonium lactate was administered to the normal as well as denervated frogs once a day for one week while the control animals received 0·5 ml of physiological saline only. The liver, kidney, brain and muscle tissues were isolated in cold and were homogenised separately in cold 0·1% cetyl trimethyl ammonium bromide (CTB) and centrifuged at 2,500 rpm for 10 minutes to remove the cell debris. The supernatant was used for enzyme assay. Arginase (L-arginine ureohydrolase) (EC 3, 5.3.1) was estimated by the method of Campbell[9] with slight modification and the urea content was estimated by the diacetyl monoxime method as described by Natelson[10].

Results

In the present study the arginase activity increased significantly in all the tissues of frog during denervation atrophy (Table I). Administration of ammonium lactate also caused enhanced activity levels of enzyme in the nm, cm and dm (Table I). However, it is the dm in which the response was highly pronounced. Induced ammonia toxicity has shown elevated arginase activity in the brain, liver and kidney tissues also (Table I). However, it is in muscle and brain that ammonia toxicity elicited greater response than liver and kidney in respect of arginase activity, though the liver and kidney are the important sites of urea cycle.

Table I

Changes in arginase activity levels in different tissues of normal (N) and denervated (D) frogs during different dosage of ammonium lactate

(Values are expressed in μ moles of urea formed/mg protein/hr)
Each value is mean ± S.D of six observations.

	Muslce			Brain		Liver		Kidney	
	nm	cm	dm	N	D	N	D	N	D
Control	0·038	0·054	0·058	0·059	0·089	1·86	2·87	1·86	2·66
SD	±0·005	±0·005	±0·009	±0·009	±0·008	±0·05	±0·09	±0·05	±0·06
% change		+42·1	+52·6		+50·8		+54·3		+43·0
Mild	0·0045[e]	0·059[e]	0·098[a]	0·060[e]	0·090[e]	2·07[a]	2·96[d]	2·63[e]	2·67[e]
SD	±0·013	±0·021	±0·008	±0·009	±0·009	±0·04	±0·08	±0·018	+0·20
% change	+18·4	+9·3	+68·9	+1·7	+1·1	+11·3	+3·1	+41·4	+0·37
Moderate	0·056[a]	0·064[d]	0·145[a]	0·071[b]	0·106[b]	2·61[a]	3·13[a]	2·57[a]	2·76[d]
SD	±0·006	±0·011	±0·019	±0·009	±0·010	±0·07	±0·13	±0·14	±0·15
% change	+47·4	+18·5	+150·0	+20·3	+19·1	+40·3	+9·1	+38·2	+3·7
Sublethal	0·059[a]	0·079[a]	0·147[a]	0·082[b]	0·124[a]	2·12[a]	3·50[a]	2·81[a]	3·13[d]
SD	±0·015	±0·015	±0·016	±0·016	±0·019	±0·04	±0·02	±0·11	±0·15
% change	+55·3	+46·3	+153·4	+38·9	+39·3	+13·9	+21·9	+51·1	+17·7

a = $P < 0.001$, *b* = $P < 0.01$, *c* = $P < 0.02$, *d* = $P < 0.05$, *e* = Not significant.

Discussion

The enzyme arginase catalyzes the hydrolysis of arginine to ornithine and urea and is present in appreciable amounts in the liver and kidney[11]. The reports on arginase activity levels in vertebrate skeletal muscle are very scanty. Narayana Reddy and Swami[12] reported significant increase of ornithine and arginine in denervated muscle of frog and speculated the possible occurrence of arginase in the denervated muscle in order to combat the ammonia toxicity[13]. Increase in ornithine concentration was also reported in the denervated rat hemidiaphragm[14]. Mc Gaughey[15] also suggested the occurrence of arginase activity in the dystrophic muscle. In agreement with the above mentioned reports, the present study also revealed the occurrence of arginase activity in the denervated muscle which is an interesting finding. Using radioisotopic techniques, Row *et al.*[16] have also observed the presence of arginase in the skeletal muscle of rat. The probable operation of urea cycle in muscle especially in dm at recognisable levels as revealed in the present study may represent an adaptive mechanism towards metabolic homeostasis in response to high ammonia concentration[2] and increase in the CO_2 storage capacity[17] of the denervated muscle. Similar enhanced arginase activity was also observed in the nm, cm and dm subjected to induced ammonia toxicity. The present study therefore brings to light that denervation and induced ammonia stress have enhanced the arginase activity for efficient disposal of ammonia in the gastrocnemius muscle of normal and denervated frogs.

The increased arginase activity in the brain, liver and kidney tissues of denervated frog as compared to the normalfrog may be due to the increased ammonia levels in these tissues following sciatectomy[2] through increased vascular transport from dm[18,19]. The intraperitoneal administration of ammonium lactate has shown an elevation in the arginase activity levels in the liver, kidney and brain tissues of both the normal and denervated frogs signalling increased ammonia detoxification by urea synthesis. It is significant to note that brain and muscle tissues of both normal and denervated frogs have registered more enhancement in enzyme activity as compared to the liver and kidney in which urea formation seems to gain precedence over glutamine formation as a means of ammonia disposal.

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