Pergamon

Phytochemistry, Vol. 46, No. 5, pp. 827–831, 1997

Printed in Great Britain
0031–9422/97 $17.00+0.00

PII: S0031–9422(97)00393–2

A SURVEY OF PLANTS FOR PRESENCE OF CHOLINESTERASE ACTIVITY

Ashu Gupta and Rajendra Gupta*

Department of Botany, University of Delhi, Delhi-110007, India

(*Received in revised form* 14 *April* 1997)

Key Word Index—Asteraceae; Euphorbiaceae; Leguminosae; Solanaceae; legumes; distribution; acetylcholine; acetylcholinesterase; anticholinesterase; cholinesterase.

Abstract—Cholinesterase (ChE) activity in plants was surveyed by employing an *in vivo* test in 106 species belonging to 53 families of Angiosperms, seven species belonging to five families of Gymnosperms and five species belonging to five families of Pteridophytes. Of the 118 species screened, ChE activity could be detected in 67 species belonging to 41 families. In plants tested positive, not all parts showed ChE activity. All the species of the families Euphorbiaceae and Leguminosae showed ChE activity. Most of the members of Solanaceae tested positive and leaves of *Physalis minima* (Solanaceae) were the richest source of ChE in plants reported thus far with ChE equal to one fifth of that present in the giant axon of squid. Most of the Asteraceae tested negative. However, the lack of detection of ChE in a plant may not imply its absence. The results confirm widespread distribution of ChE in plant kingdom and identify rich sources of ChE to be used as model experimental systems. © 1997 Elsevier Science Ltd

INTRODUCTION

Acetylcholine (ACh) the well-known neurotransmitter and the enzymes of its metabolism—cholinesterase (ChE) and choline acetyltransferase—have been reported to be present at several non-neuronal locations in animals [1–3] and plants [4–7]. However, the physiological role of ACh in non-nervous systems is far from clear [1–3, 6]. In order to understand the role of ACh system in plants, it is desirable to know the distribution of ACh and enzymes of its metabolism in different plant parts. Plants that are particularly rich in various components of the ACh system should also be identified for use as model experimental materials. The results of a survey for ChE in various parts of 118 plant species are presented here.

RESULTS AND DISCUSSION

Acetylthiocholine iodide (ATChI) hydrolysis that could be completely inhibited by 25 μM neostigmine (Nst) was used as a marker for the presence of ChE. Neostigmine is a potent anti-ChE agent in animals [1] and plants [5, 8]. Of the 118 plant species screened (Table 1) ChE could be detected in 67 species only (three out of five Pteridophytes, six out of seven Gymnosperms and 58 out of 106 Angiosperms). All leguminous plants contained ChE. No legume tested thus far in any survey [9–11] has shown lack of ChE. Besides legumes, all species of the family Euphorbiaceae tested positive, though the activity of ChE was very low. The Solanaceae emerged as an important and rich source of ChE. Five out of six Solanaceae members tested positive and two of these, *Physalis minima* and *Datura innoxia*, had higher ChE activity than reported thus far from any plant (2717 and 2005 pmol ATChI hydrolysed s^{-1} g^{-1} fr. wt, respectively) which is about one fifth of that present in the giant axon of squid [see 12] and therefore can be exploited as important sources of ChE. Interestingly, Solanaceae is known to contain anti-ChEs α-chaconine, solanine [13, 14], as well as many compounds such as nicotine, scopolamine, atropine and hyoscyamine which affect the ACh system in animals [15]. 12 out of 13 Asteraceae members tested did not have any detectable ChE activity. Since one of these Asteraceae member *Parthenium hysterophorus* is known to contain anti-ChE principles [16] while another, *Chrysanthemum cinerariaefolium*, contains pyrethrin, an insecticide having neuroblocking activity [17], it would be interesting to search for new anti-ChE compounds in Asteraceae members.

Of 118 species screened we have not detected ChE activity in 51 species. Even in plants tested positive, not all parts of the plant showed ChE activity. Lack of demonstrable ChE may not imply its absence for

* Author to whom correspondence should be addressed

Table 1. Neostigmine inhibited hydrolysis of ATChI in plants

Family	Plant	ATChI hydrolysed* (pmol s^{-1} g^{-1} fr. wt) Leaf	Stem/ Branch	Root
Angiosperms				
Acanthaceae	*Justicia gendarussa* Burm. f.	674	511	ND
	Peristrophe paniculata (Forsk.) Brummitt	ND	ND	ND
Agavaceae	*Dracaena deremensis* Engl.	ND	?	?
Aizoaceae	*Mesembryanthemum crystallinum* L.	ND	ND	ND
	Trianthema portulacastrum L.	264	141	270
Anacardiaceae	*Mangifera indica* L.	ND	?	?
Annonaceae	*Polyalthia longifolia* Thw.	ND	?	?
Apiaceae	*Daucus carota* L.	?	?	ND
Apocynaceae	*Carissa carandus* L.	ND	?	?
	Catharanthus roseus (L.) G. Don	71	ND	ND
	Tabernaemontana divaricata (L.) R. Br.	ND	ND	?
	Thevetia peruviana (Pers.) Merr.	ND	ND	?
Araceae	*Colocasia esculenta* (L.) Schott	ND	?	?
Arecaceae	*Caryota urens* L.	ND	?	?
	Livistona chinensis R. Br.	202	?	?
Asclepiadaceae	*Calotropis procera* (Ait.) R. Br.	ND	ND	?
Asparagaceae	*Asparagus racemosus* Willd.			
	Seedling	319	ND	ND
	Old plant	182	?	?
	Ruscus aculeatus L.	72	ND	?
Asteraceae	*Ageratum conyzoides* L.	ND	ND	ND
	Bidens biternata (Lour.) Merr. & Sherff	ND	ND	ND
	Chrysanthemum cinerariaefolium Vis.	139	ND	?
	Eclipta prostrata (L.) L.	ND	ND	ND
	Galinsoga quadriradiata Ruiz & Pav.	ND	ND	ND
	Gnaphalium indicum L.	ND	?	ND
	Helianthus annuus L.	ND	ND	?
	Parthenium hysterophorus L.	ND	ND	ND
	Sonchus oleraceus L.	ND	ND	ND
	Tagetes erecta L.	ND	ND	?
	Tridax procumbens L.	ND	ND	?
	Vernonia conyzoides DC.	ND	ND	ND
	Vernonia elaeagnifolia DC.	ND	ND	?
Bombacaceae	*Bombax ceiba* L.			
	Seedling	100	ND	?
Brassicaceae	*Coronopus didymus* (L.) Sm.	ND	?	ND
	Sisymbrium irio L.	ND	?	ND
Burseraceae	*Commiphora wightii* (Arnott.) Bhandari	535	?	?
Cannabaceae	*Cannabis sativa* L.	ND	142	92
Cannaceae	*Canna indica* L.	ND	ND	?
Caprifoliaceae	*Sambucus nigra* L.	?	98	?
Caricaceae	*Carica papaya* L.	482	?	?
Caryophyllaceae	*Stellaria media* (L.) Vill.	231	ND	?
Ceratophyllaceae	*Ceratophyllum demersum* L.	ND	ND	?
Combretaceae	*Quisqualis indica* L.	133	?	?
Commelinaceae	*Tradescantia virginiana* L.	72	ND	?
Convolvulaceae	*Ipomoea nil* (L.) Roth	ND	83	?
	Evolvulus nummularius L.	ND	ND	ND
Crassulaceae	*Kalanchoe pinnata* (Lamk.) Pers.	351	?	?
Cucurbitaceae	*Coccinia cordifolia* (L.) Cogn.	ND	ND	?
Euphorbiaceae	*Acalypha indica* L.	?	63	76
	Codiaeum variegatum Blume	123	?	?
	Codiaeum variegatum Blume	572	?	?
	Codiaeum variegatum Blume	ND	?	?
	Codiaeum variegatum Blume	511	?	?
	Euphorbia hirta L.	159	ND	329
	Euphorbia milii Ch. des Moul.	61	?	?
	Euphorbia neriifolia L.	?	260	?

Table 1. (*Continued*)

Family	Plant	Leaf	ATChI hydrolysed* (pmol s^{-1} g^{-1} fr. wt) Stem/ Branch	Root
	Euphorbia pulcherrima Willd. ex Klotz.	76	ND	152
	Jatropha integerrima Jacq.	92	67	?
	Phyllanthus fraternus Webster	305	163	235
	Putranjiva roxburghii Wall.	264	ND	?
	Ricinus communis L.	84	ND	ND
Hydrocharitaceae	*Hydrilla verticillata* (L.f) Royle	ND	96	?
Lamiaceae	*Coleus blumei* Benth.	235	ND	?
	Ocimum sanctum L.	ND	?	?
Leguminosae	*Aeschynomene indica* L.	550	?	?
	Caesalpinia pulcherrima (L.) Sw.	235	ND	?
	Cassia occidentalis L.	123	127	397
	Crotalaria juncea L.	353	ND	?
	Leucaena leucocephala (Lamk.) Wit	170	?	?
	Psophocarpus tetragonolobus (L.) DC.	288	161	?
	Sesbania sesban (L.) Merr.	206	63	121
Magnoliaceae	*Magnolia grandiflora* L.	ND	?	?
Malvaceae	*Gossypium herbaceum* L.	119	215	?
	Hibiscus rosa-sinensis L.	ND	ND	?
Meliaceae	*Melia azedarach* L.	ND	ND	ND
Moraceae	*Ficus benghalensis* L.	ND	?	?
	Ficus elastica Roxb.	Y ND O 61	196	?
	Ficus krishnae C.DC.	286	ND	?
	Ficus racemosa L.	ND	?	?
	Ficus religiosa L.	ND	ND	?
Musaceae	*Musa paradisiaca* L.	ND	?	?
Myrtaceae	*Callistemon lanceolatus* DC.	102	ND	?
	Psidium guajava L.	143	?	?
Nyctaginaceae	*Boerhavia diffusa* L.	208	ND	?
	Bougainvillea glabra Choisy	ND	145	?
Oxalidaceae	*Oxalis corniculata* L.	?	ND	ND
Papaveraceae	*Papaver somniferum* L.	151	ND	ND
	Argemone mexicana L.	?	ND	?
Piperaceae	*Piper betle* L.	259	?	?
Poaceae	*Cynodon dactylon* (L.) Pers.	ND	79	ND
Polygonaceae	*Antigonon leptopus* Hook. & Arn.	ND	ND	?
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	ND petiole ND	92	235
Portulacaceae	*Portulaca quadrifida* L.	ND	ND	ND
Proteaceae	*Grevillea robusta* A. Cunn.	ND	ND	?
Punicaceae	*Punica granatum* L.	116	?	?
Rubiaceae	*Ixora coccinea* L.	ND	?	?
Rutaceae	*Citrus aurantiifolia* (Christm.) Swingle	762	347	?
	Vitis vinifera L.	235	?	?
Scrophulariaceae	*Mazus pumilus* (Burm.f.) Steen.	102	ND	ND
	Verbascum chinense (L.) Santapau	ND	ND	ND
Solanaceae	*Datura innoxia* Mill.			
	Young plant	2005	705	408
	Old plant	1263	100	?
	Nicotiana plumbaginifolia Viv.	ND	ND	ND
	Nicotiana rustica L.			
	Young plant	245	?	ND
	Physalis minima L.			
	Young plant	1716	1481	163
	Old plant	2717	1123	?
	Solanum nigrum L.	82	ND	ND
	Withania somnifera (L.) Dunal	1451	548	ND

Table 1. (*Continued*)

Family	Plant	Leaf	ATChI hydrolysed* (pmol s^{-1} g^{-1} fr. wt) Stem/Branch	Root
Tiliaceae	*Corchorus aestuans* L.	92	ND	?
Tropaeolaceae	*Tropaeolum majus* L.	106	ND	188
Verbenaceae	*Lantana camara* L.	ND	ND	?
	Nyctanthes arbor-tristis L.	ND	ND	?
Gymnosperms				
Cupressaceae	*Biota orientalis* (D.Don) Endl.	74	?	?
Cycadaceae	*Cycas revoluta* Thunb.	241	?	?
Ephedraceae	*Ephedra foliata* Boiss.	ND	?	?
Ginkgoaceae	*Ginkgo biloba* L.	231	?	?
Zamiaceae	*Dioon edule* Lindl.	262	?	?
	Dioon spinulosum Dyer.	176	?	?
	Zamia furfuracea L.f.	Y ND O 175	?	?
Pteridophytes		Frond	Stalk	Rhizoid
Adiantaceae	*Adiantum capillus-veneris* L.	ND	ND	ND
Equisetaceae	*Equisetum ramosissimum* Desf.	?	ND	86
Oleandraceae	*Nephrolepis biserrata* (Sw.) Schott	176	155	?
Pteridaceae	*Pteris multifida* Poir.	388	ND	82
Salviniaceae	*Salvinia natans* (L.) All.	ND	?	ND

*Values of controls containing anti-ChE Nst were subtracted from the values in tests. Each value is a mean of three replicate determinations.

ND, Not detected (less than 61 pmol s^{-1} g^{-1} fr. wt); ?, Not tested; Y, young; O, old.

several reasons. The method used may not be sensitive enough to detect low levels of ChE present in some plants. The presence of a high concentration of thiols in certain plants might interfere with the Ellman's spectrophotometric test [18] employed in present study. The plant might contain a factor/factors that decolorizes the reaction product in Ellman's spectrophotometric test [9]. There might be present some natural ChE inhibitor in plants [15, 16]. Plant part other than those tested might possess ChE activity. It is also quite possible that ChE activity is absent at the physiological stage at which plant was screened, though it may be present at a different physiological stage/stages.

The results of the present survey as well as more work in progress in our laboratory and the earlier studies [9–11] show that ChEs are widespread in plants. It appears *prima facie* that the Leguminosae and Solanaceae that have high concentration of ChE also have maximum number of edible plants. Since plants and animals have co-evolved, there may be a relationship between evolution of eating habits and presence or absence of ChE/anti-ChE in plants because anti-ChEs are neurotoxins. The possibility of employing ChE containing plants for monitoring exposure to anti-ChE agents used in chemical warfare and during emergencies arising out of industrial leakage of anti-ChE pesticides should also be explored. Plants or plant parts showing a lack of detectable ChE should be searched for the presence of naturally occurring anti-ChE compounds for use as bioinsecticides and in therapeutics.

EXPERIMENTAL

The plants growing in and around the Department of Botany, University of Delhi, were used in the present study. Identification and nomenclature of plants was based on standard texts [19–23]. The identities of plants were further confirmed by matching with the specimen in the Delhi University Herbarium.

Cholinesterase activity was measured spectrophotometrically by employing a minor modification of the method of ref. 18. In the original method, the time course of the enzymatic activity was recorded, whereas in the present study the enzyme activity was tested after a fixed time of 30 min for each sample. It was ascertained that the enzyme activity remains linear at least for 30 min even in samples containing high ChE activity. The test is based on hydrolysis of acetylthiocholine, a thiol analogue of ACh, to acetate and thiocholine, and reaction of the thiocholine with sulphydryl detection reagent 5:5′-dithiobis-2-nitrobenzoate (DTNB) to yield yellow coloured anion of 5-thio-2-nitrobenzoate having molar absorbance coefficient equal to 1.36×10^4 [18]. Besides thiocholine produced as a result of enzymatic hydrolysis of ATChI, some other factors may also contribute to the yellow colour produced in the test, e.g. autohydrolysis of ATChI to thiocholine, other thiol compounds including sulphydryl groups of proteins or some yellow coloured pigments leached out from the sliced plant material. To check for the above factors, *A* was also recorded in controls containing the specific anti-ChE compound, Nst. Data of control sets was deducted from the corresponding test data. Experiments were carried out at 30°. The reaction medium in a final vol. of 5 ml contained K-Pi buffer pH 8, 0.1 M, DTNB 0.1 mM, ATChI 1 mM and 100 mg plant material (2 mm slices). Controls were preincubated with anti-ChE Nst 25 μM for 30 min before addition of ATChI. The incubation medium was changed before adding ATChI in some plants that showed an intense yellow colour in the incubation medium due to presence of thiol compounds. 30 min after the addition of ATChI, the contents of tubes were filtered through double layer of cheese cloth and *A* recorded at 412 nm. Each assay consisted of three replicates.

Acknowledgements—We are grateful to the University Grants Commission (UGC), New Delhi, for a research grant (RG) and a Junior Research Fellowship (AG). We thank Professors C. R. Babu and K. M. M. Dakshini, Botany Department, Delhi University for help in identification of some plants, Dr H. R. Das, Centre for Biochemical Technology, Mall Road, Delhi, for gift of ATChI, DTNB and Nst for starting the work, Prof. S. C. Gupta for his interest and use of spectrophotometer and Prof. P. S. Ganapathy for the benefit of discussion.

REFERENCES

1. Silver, A., *The Biology of Cholinesterases. North-Holland Research Monographs, Frontiers of Biology*, Vol. 36. North-Holland Publishing Company, Amsterdam, 1974.
2. Sastry, B. V. R. and Sadavongvivad, C., *Pharmacological Reviews*, 1979, **30**, 66.
3. Quinn, D. M., Balasubramanian, A. S., Doctor, B. P. and Taylor, P., eds, *Enzymes of the Cholinesterase Family*. Plenum Press, New York, 1995.
4. Whittaker, V. P., in *Handbuch der Experimentellen Pharmakologie*, Vol. 15, *Cholinesterases and Anticholinesterase Agents*, ed. G. B. Koelle. Springer, Berlin, 1963, p. 1.
5. Fluck, R. A. and Jaffe, M. J., *Current Advances in Plant Science*, 1974, **5**(11), 1.
6. Hartmann, E. and Gupta, R., in *Plant Biology*, Vol. 6, *Second Messengers in Plant Growth and Development*, eds. W. F. Boss and D. J. Morre. Alan R. Liss, New York, 1989, p. 257.
7. Tretyn, A. and Kendrick, R. E., *The Botanical Review*, 1991, **57**, 33.
8. Gupta, R. and Maheshwari, S. C., *Plant and Cell Physiology*, 1980, **21**, 1675.
9. Fluck, R. A. and Jaffe, M. J., *Phytochemistry*, 1974, **13**, 2475.
10. Maheshwari, S. C., Gupta, R. and Gharyal, P. K., in *Recent Developments in Plant Sciences*, ed. S. P. Sen. Today and Tomorrow's Printers and Publishers, New Delhi, 1982, p. 145.
11. Miura, G. A., Broomfield, C. A., Lawson, M. A. and Worthley, E. G., *Physiologia Plantarum*, 1982, **56**, 28.
12. Dettbarn, W. D., *Nature*, 1962, **194**, 1175.
13. Alozie, S. O., Sharma, R. P. and Salunkhe, D. K., *Journal of Food Biochemistry*, 1978, **2**, 259.
14. Patil, B. C., Sharma, R. P., Salunkhe, D. K. and Salunkhe, K., *Food and Cosmetics Toxicology*, 1972, **10**, 395.
15. Long, J. P., in *Handbuch der Experimentellen Pharmakologie*, Vol. 15, *Cholinesterases and Anticholinesterase Agents*, ed. G. B. Koelle. Springer, Berlin, 1963, p. 374.
16. Hariharan, N. V., Nandakumar, N. V. and Murthy, N. V. A., *Comparative Physiology and Ecology*, 1983, **8**, 53.
17. Matsuda, K., Nishikawa, M., Hamada, M., Nishimura, K. and Fujita, T., *Pesticide Biochemistry and Physiology*, 1991, **41**, 170.
18. Ellman, G. L., Courtney, K. D., Andres, V., Jr. and Featherstone, R. M., *Biochemical Pharmacology*, 1961, **7**, 88.
19. Maheshwari, J. K., *The Flora of Delhi*, Council of Scientific and Industrial Research, New Delhi, 1963.
20. Maheshwari, J. K., *Illustrations to the Flora of Delhi*, Council of Scientific and Industrial Research, New Delhi, 1966.
21. Bailey, L. H., *Manual of Cultivated Plants*, The Macmillan Company, New York, 1949.
22. Bennet, S. S. R., *Name Changes in Flowering Plants of India and Adjacent Regions*, Triseas Publishers, Dehra Dun, 1987.
23. Willis, J. C., *A Dictionary of the Flowering Plants and Ferns*, Cambridge University Press, Cambridge, 1966.