

BRIEF COMMUNICATION

An Acetylcholine-like Substance in *Porophyllum lanceolatum*

E. W. HORTON and G. M. FELIPPE

Department of Pharmacology and Department of Botany, University of Edinburgh

Received October 10, 1972

Abstract. Evidence for the occurrence of a substance closely resembling acetylcholine in *Porophyllum lanceolatum* DC is presented. Its concentration in terms of acetylcholine estimated by biological assay was $0.1 \pm 0.02 \mu\text{mol g}^{-1}$ fresh weight of leaf.

Porophyllum lanceolatum DC is a member of the *Compositae* occurring in the 'Cerrado' vegetation of Brazil. In some regions of that country it is popularly believed that infusions of the plant are an effective remedy for hypertension. This investigation was initially undertaken to test the hypothesis that a substance of potential medicinal importance might be present. A substance with similar properties to acetylcholine has been identified in large quantities from *Porophyllum lanceolatum*.

Plants of *P. lanceolatum* were grown from seeds in a heated glass house. An aqueous extract was prepared by maceration of leaves in ten volumes of water, followed by filtration. The filtrate was concentrated to about half its volume by evaporation under reduced pressure and sufficient sodium chloride was added to make the solution isotonic to blood. Biological assays were performed by standard pharmacological techniques (BURN 1952).

The aqueous extract was compared with acetylcholine on five biological preparations (Table 1). The responses to the extract were qualitatively similar to those of acetylcholine. The log dose response curves were parallel and the responses to both acetylcholine and the extract were abolished by atropine sulphate but not by mepyramine maleate. There was good quantitative agreement when the concentration of acetylcholine in the extract was determined by four different assays.

The active principle resembled acetylcholine in its stability and solubility (Table 1). Like acetylcholine it was unstable in blood but was not inactivated by trypsin or chymotrypsin. Extracts of leaves from different plants were assayed. The mean content ($\pm$ s.e.m.) of acetylcholine was found to be $0.1 \pm 0.02 \mu\text{mol g}^{-1}$ ($n = 13$).

Chemical evidence is needed for conclusive identification of acetylcholine in the leaves of *Porophyllum lanceolatum*. The present pharmacological data indicate that the substance is either acetylcholine or a very closely related choline ester.

The detection of acetylcholine in *P. lanceolatum*, a plant in which phytochrome-mediated responses have been shown to occur (FELIPPE and LUCAS

TABLE 1

PROPERTIES OF THE SUBSTANCE IN THE LEAVES of *Porophyllum lanceolatum*
In all tests the response to the principle was identical with that to acetylcholine

Test	Response	Estimated acetylcholine concentration [$\mu\text{mol g}^{-1}$ leaf]
1. Pharmacological actions		
(a) Cat arterial blood pressure	Lowered, especially diastolic pressure; unaffected by bilateral vagotomy or hexamethonium ($28 \mu\text{mol}$); abolished by atropine ($1.4 \mu\text{mol}$)	0.15
(b) rat isolated fundal strip	Contraction, cumulative log dose response curves parallel; abolished by atropine ($3 \times 10^{-6}\text{M}$)	0.14
(c) rabbit isolated jejunum	Contraction, abolished by atropine ($1.5 \times 10^{-6}\text{M}$)	0.15
(d) cat tracheal chain	Contraction	0.14
(e) guinea-pig isolated ileum	Contraction; abolished by atropine ($1.5 \times 10^{-7}\text{M}$) but not by mepyramine ($5 \times 10^{-6}\text{M}$)	
2. Stability	5% of biological activity remained after 15 min at pH 12.5 and 20°C 100% of biological activity remained after 60 min at pH 7.0 and 99°C	
3. Enzymes	Inactivated by incubation with cat blood for 5 min at 37°C	
4. Solubility	Petroleum spirit — insoluble Ethyl acetate — slightly soluble Methanol — moderately soluble	

1971; FELIPPE *et al.* 1971a, 1971b) suggests that this species would be a useful tool for studies on the relationship between phytochrome and acetylcholine (JAFFE 1970). Plants grow rapidly in the glasshouse and within a month from planting flowering can be induced by short-day treatment.

References

- BURN, J. H.: Practical Pharmacology. — Blackwell, Oxford 1952.
 FELIPPE, G. M., GIULIETTI, A. M., LUCAS, N. M. C.: Estudos de germinacao em *Porophyllum lanceolatum* DC. I. Efeito de luz, temperatura e fotoperiodo. — *Hoehnea* 1 : 1—9, 1971a.
 FELIPPE, G. M., LUCAS, N. M. C.: Estudos de germinacao em *Porophyllum lanceolatum* DC. II. Efeito de luz vermelha, GA_3 e CCC. — *Hoehnea* 1 : 11—19, 1971.
 FELIPPE, G. M., LUCAS, N. M. C., GIULIETTI, A. M.: Estudos de floração em *Porophyllum lanceolatum* DC. I. Efeito do fotoperiodo na floração. — *Hoehnea* 1 : 21—27, 1971b.
 JAFFE, M. J.: Evidence for the regulation of phytochrome-mediated processes in bean roots by the neurohumor, acetylcholine. — *Plant Physiol.* 46 : 768—777, 1970.