Isoamylamine as the Possible Neuroactive Metabolite of L-Leucine

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In previous papers, we reported that a neuroactive substance was isolated from the blood of DDT-poisoned silkworm larvae, *Bombyx mori*, and was identified as L-leucine.^{1,2)} L-Leucine increased the spontaneous discharge on the isolated nerve cord of American cockroach, *Periplaneta americana*, whereas D-leucine did not show such an activity. Sternburg *et al.* have reported that electrical stimulation or DDT-treatment on the isolated nerve cord caused the release of a neuroactive substance into the perfusing saline.^{3,4)} This paper describes the release of leucine or its metabolite from the nerve cord of American cockroach by both DDT-treatment and electrical stimulation, the presence of leucine decarboxylase in the nerve tissues, and the neuroactivity of isoamylamine, the possible decarboxylation metabolite of leucine.

A 10 μ l dose of L-leucine-4,5-³H (1 mCi/ml) was administered daily by injection into the abdominal cavity of each adult male cockroach for four days (the total amount administered was 2 m μ mole). On the fifth day, the thoracic-abdominal nerve cords were isolated from the cockroaches. The distribution of L-leucine incorporated was measured by counting scintillation after washing the nerve cord with a flowing physiological saline solution for one hour. Radioactivity per tissue weight was 60% more in the ganglion than in the axon. In order to measure the release of radioactivity from the nerve cord, it was immersed in physiological saline flowing at a constant speed of 1 ml/min in a small chamber (2.5 ml) and was subjected to treatment with a DDT-saline solution (10^{-4} M) containing 0.5%

ethanol or with electrical stimulation (100 stimulations per sec., 1.5 V) after perfusing with the saline for one hour. A stimulating Ag-AgCl electrode (100 μ m in diameter) was placed between the prothoracic and mesothoracic ganglia and an indifferent electrode was placed beside the mesothoracic ganglion near the stimulating electrode. Radioactivity released into the flowing saline was measured by collecting the saline into a vial during 20 min periods, concentrating them to 0.5 ml and adding a scintillation solution.

The release of radioactivity from the nerve cord was accelerated by both the DDT-treatment and electrical stimulation. Forty minutes treatment with the DDT solution after 60 min perfusion increased the release of radioactivity about 70% and the electrical stimulation caused about 35% increase, in comparison with a control perfusion with 0.5% ethanol in the saline, in which radioactivity released was 200 ± 30 cpm. The released substance was submitted to paper chromatography (butanol: acetic acid: water = 4: 1: 5) and radioactivity was found in the regions of *Rf* 0~0.2 and 0.6~0.75. The latter corresponds to leucine (*Rf* 0.62) or its possible metabolite isoamylamine (*Rf* 0.68). Similar experiments performed with the nerve cord which had incorporated the related non-neuroactive amino acid isoleucine-(U)-³H showed that radioactivity was released from the nerve cord by the DDT-treatment, but was not by the electrical stimulation.

We studied then the metabolism of leucine in the nerve tissues. Leucine was decarboxylated by the action of a decarboxylase present in the nerve cord of American cockroach. An acetone powder of the nerve cords from the cockroaches was used as the enzyme source and DL-leucine-1-¹⁴C (5×10^{-2} μ mole) as the substrate. Liberated ¹⁴CO₂ was absorbed into an ethanolamine-methylcellosolve mixture permeated to a piece of filter paper hung in a stoppered reaction vessel. In the presence of pyridoxal phosphate, ¹⁴CO₂ was liberated as shown in Table I. Thus it is reasonable to presume that leucine is transformed into

TABLE I. DECARBOXYLASE ACTIVITY OF NERVE TISSUE TOWARD LEUCINE

Reaction mixture: Tris-HCl (pH 7.4) 100 mM, pyridoxal phosphate 0.12 μ mole, enzyme preparation 10 mg, final volume 0.3 ml. The mixture was incubated at 37°C for 30 min. Back ground (108 cpm) was deducted.

No. of experiment	$^{14}\text{CO}_2$ liberated	
	With enzyme	Without enzyme
1	5824	711
2	4808	632

FIG. 1. Activation of Spontaneous Discharge on the Isolated Nerve Cord of *Periplaneta americana* by Isoamylamine at 10^{-5} M.

isoamylamine in the nerve tissues through the action of the decarboxylase.

The neuroactivity of isoamylamine was then assayed by recording the spontaneous discharge on the isolated abdominal nerve cord. The amine caused an extreme increase in the spontaneous discharge of the nerve cord as

shown in Fig. 1. Contrasting with the slow onset by L-leucine (8 min), the action of isoamylamine appeared rapidly and the discharge reached the maximum at about 4.5 min after the treatment. At higher concentration (10^{-4} M) the discharge was blocked after it reached the maximum. The rapid onset of the spontaneous discharge by isoamylamine (within 3 min) indicates that the amine exerts the effect directly on the nerve tissue. The slow action of L-leucine and the decarboxylase activity of the nerve tissues towards the amino acid suggest that isoamylamine is a real neuroactive principle. Although isoamylamine could be produced by the decarboxylation of D-leucine too, the latter is neurophysiologically inactive. This may be attributable to the substrate specificity of the decarboxylase for the L-form of the amino acid.

Isoamylamine has weak insecticidal activity when injected into houseflies, a dose of 15 μ g per insect resulting in almost instant knock-down followed by 30% of the individuals recovering.⁵⁾ This response is, however, not specific for isoamylamine since some other aliphatic amines produce similar effects on houseflies.⁵⁾

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REFERENCES

- 1) S. Tashiro, E. Taniguchi and M. Eto, *Science*, **175**, 448 (1972).
- 2) S. Tashiro, E. Taniguchi and M. Eto, *Agr. Biol. Chem.*, **36**, 2465 (1972).
- 3) J. Sternburg, S. C. Chang and C. W. Kerns, *J. Econ. Entomol.*, **52**, 1070 (1959).
- 4) J. Sternburg, *J. Agr. Food Chem.*, **8**, 257 (1960).
- 5) J. E. Casida and J. Engel, Personal communication (1974).