High-Performance Liquid Chromatographic Analysis of Monoamines and Some of Their \( \gamma \)-Glutamyl Conjugates Produced by the Brain and Other Tissues of *Helix aspersa* (Gastropoda)

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SUMMARY

1. Earlier reports from this and other laboratories have indicated that wide variations exist in estimates of the concentrations of norepinephrine in the brain and heart of the snail *Helix aspersa*. This is a report on investigations of norepinephrine concentrations in *Helix aspersa* tissues using high-performance liquid chromatography with electrochemical detection. In addition, the effects of treatment with some amino acid precursors or enzyme inhibitors on the concentrations of norepinephrine, dopamine, 5-hydroxytryptamine, and some of their metabolites were investigated.

2. The levels of norepinephrine in the brain were low (46 ng/g) in comparison to dopamine (2.1 \( \mu \)g/g) and 5-hydroxytryptamine (2.6 \( \mu \)g/g). Epinephrine was not observed in either snail heart or snail nervous tissue.

3. Administration of L-3,4-dihydroxyphenylalanine resulted in elevated snail brain dopamine, while 3,4-dihydroxyphenylserine treatment increased norepinephrine. Treatment with blockers of tyrosine hydroxylase and aromatic-L-amino acid decarboxylase reduced dopamine concentrations without affecting 5-hydroxytryptamine.

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4. The dopamine metabolite 3,4-dihydroxyphenylacetic acid was observed only after administration of L-3,4-dihydroxyphenylalanine or dopamine and then only in very small amounts. At no time was the dopamine metabolite homovanillic acid or the 5-hydroxytryptamine metabolite 5-hydroxyindoleacetic acid observed in brain, heart, or whole-body extracts of the snail.

5. Incubation of nervous tissue with either dopamine or 5-hydroxytryptamine resulted in the production of electrochemically active metabolites which were identified by oxidation characteristics and cochromatography with synthesized standards as the $\gamma$-glutamyl conjugates of the amines. Treatment of snails with 5-hydroxytryptamine or dopamine also resulted in the production of $\gamma$-glutamyl conjugates.

6. The present experiments show that great care must be exercised when measuring monoamines and their metabolites in gastropod tissues by high-performance liquid chromatography with electrochemical detection.

INTRODUCTION

In recent years gastropods, in particular *Aplysia californica*, *Helix pomatia*, and *Helix aspersa*, have been used as models in the study of the aminergic pharmacology of mollusk heart contraction, learning behavior, and general physiology (Kerkut and Walker, 1961; Mirolli, 1968; Wollemann and S-Rozsa, 1975). Since the importance of monoamines in the control of many dynamic events concerned with mollusk physiology has been demonstrated, many researchers have attempted to elucidate the metabolic pathways concerned with amine synthesis and catabolism in these animals. This has led to a number of publications concerning the levels of norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT), octopamine (OA), epinephrine (EPI), and their metabolites in gastropod tissues (Osborne and Cottrell, 1970; Juorio and Killick, 1972; Goldman and Schwartz, 1977; Straub and Kuhlmann, 1984; Franchini et al., 1985; Ottaviani et al., 1988). Unfortunately substantially different results, both within and between species, have been reported. In particular, the reported concentrations of NE and EPI in the brain and the occurrence of 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5HIAA), and monoamine oxidase (MAO) activity are variable. Very low levels of NE have been reported for gastropod nervous tissue [80 ng/g, *Helix aspersa* (Osborne and Cottrell, 1970); 70–80 ng/g, *Helix pomatia* (Juorio and Killick, 1972); low or undetectable, several species (Straub and Kuhlmann, 1984)]. This contrasts with other reports where NE was determined to occur in high amounts [420 ng/g, *Helix aspersa*, (Osborne, 1984); 765 and 1040 ng/g, *Helicella virgata* (Franchini et al., 1985; Ottaviani et al., 1988)]. In addition, Osborne (1984) and Ottaviani et al. (1988) report EPI concentrations of 380 and 70 ng/g, respectively, whereas this substance remained undetectable to other investigators (see Ottaviani et al., 1988). Indeed, Ottaviani et al. (1988) report concentrations of 3,4-dihydroxyphenylalanine (DOPA), $L$-normetanephrine, and $L$-metanephrine in the range of 3 to 4 $\mu$g/g in the brain of *Helicella virgata*. These levels are far in excess of those determined for any other gastropod tissue.