The Effect of Fenfluramine, 2,5-Dimethoxy-4-methyl Amphetamine (DOM) and D-Amphetamine on the Concentration of Serotonin and Some Free Amino Acids in the Suboesophageal Ganglia of *Helix pomatia*

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The effects of a number of phenylethylamines on the metabolism of brain biogenic amines have been studied with the aim of differentiating neurochemically between those drugs having a stimulant profile from those which are hallucinogenic [1–5]. The present study was undertaken to see if some of these drugs had a similar effect on the metabolism of serotonin in the oesophageal ganglia of the snail so that an assessment could be made of the possible usefulness of this *in vitro* preparation in the initial screening of centrally acting drugs for their neurochemical action. In this study, the effects of the stimulant drug, D-amphetamine have been compared with those of the hallucinogen DOM and the non-stimulant, non-hallucinogen fenfluramine. The suboesophageal ganglion preparation of *Helix pomatia* was selected because other studies have shown that it is particularly sensitive to drug effects [6]. Furthermore the changes found following the administeration of a number of neuropharmacological agents were qualitatively similar to those found in mammals [6, 7]. The changes produced by such drugs in the single ganglia preparation can be quantified using the sensitive dansyl chloride procedure described in detail by Briel and Neuhoff [8], Osborne [11] and Neuhoff [12].

Suboesophageal ganglia were dissected from *Helix pomatia*, allowed to equilibrate for 10 min in snail saline [9] and then placed for 1 hour either in a solution of snail saline (controls) or in saline containing 1 μg/ml of fenfluoramine, DOM or D-amphetamine. The amines and amino acids from each ganglion preparation were then extracted by homogenizing in 0.01 M sodium bicarbonate solution (pH 10) and treated with [^{14}C]dansyl chloride (specific activity 49 mCi/mmol). The [^{14}C]dansylated substances were then chromatographed on 3×3 cm polyamide layers. After visualisation of the spots by UV light, the individual spots were removed and the radioactivity assessed using a liquid scintillation spectrometer. This method has been described in detail elsewhere [8, 10–12]. The results are expressed as the dpm of the dansylated product as a percentage of total radioactivity of all the products separated.

The results of this investigation are shown in Table I. DOM caused a significant increase in the concentration of serotonin and a slight decrease in that of 5-hydroxyindolacetic acid. These changes are qualitatively similar to those found when the drug is administered to that rat [5] and suggest that the drug decreases the turnover of this amine in both the rat and snail nervous system. DOM also decreased the concentration of γ-aminobutyric acid in the snail ganglia; this inhibitory transmitter is also reduced in rat brain by DOM [5].

Table I. Effect of fenfluramine, DOM and D-amphetamine on serotonin, 5-hydroxyindolacetic acid and some amino acid levels in the isolated suboesophageal ganglia of *Helix pomatia*.

Substance	Controls	Fenfluramine	DOM	D-Amphetamine
Serotonin	0.73 ± 0.10	**1.19 ± 0.13 ***	1.08 ± 0.12 *	0.69 ± 0.12
5-Hydroxyindolacetic acid	0.42 ± 0.03	0.46 ± 0.05	0.30 ± 0.02 *	0.41 ± 0.04
Tryptophan	0.76 ± 0.09	0.66 ± 0.07	0.71 ± 0.08	0.68 ± 0.09
Tyrosine	1.09 ± 0.09	1.01 ± 0.08	1.19 ± 0.10	1.13 ± 0.13
γ-Aminobutyric acid	0.50 ± 0.03	0.49 ± 0.02	0.41 ± 0.01 **	0.42 ± 0.03
Methionine	0.95 ± 0.10	1.08 ± 0.19	1.33 ± 0.14 *	1.29 ± 0.09 *

Results are expressed as Mean ± S.E.M. ($N = 10$) radioactivity (dpm) of the individual dansylated product as percentage of the total radioactivity of the total products determined. * $p<0.05$; ** $p<0.1>0.05$.
The following substances were also determined but were unchanged by the drugs: glutamic acid, aspartic acid, alanine, ethanolamine, ornithine, lysine, leucine, isoleucine, valine, proline, phenylalanine, histidine, cystine, serine, threonine, cystein and 5-hydroxyindole.

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Fenfluoramine was also found to significantly increase the concentration of serotonin in the snail ganglia without appreciably affecting that of 5-hydroxyindolacetic acid. This effect on serotonin is dissimilar to that found in the rat brain after fenfluramine administration as it has been reported that the levels of this amine are reduced by the drug [13].

D-amphetamine did not significantly affect the relative concentrations of either serotonin or 5-hydroxyindolacetic acid in the snail ganglia, a finding which is similar to that observed for the action of this drug on the rat brain [1].

Of the remaining parameters which were studied in the snail ganglia, only methionine was significantly affected by any of the drugs. Both D-amphetamine and DOM decreased the relative concentration of this amino acid. While the significance of this effect must await further investigation it is possible that the decreased methionine levels are a reflection of increased transmethylation processes.

These results suggest that the isolated snail ganglion preparation may be useful in the preliminary screening of the effects of centrally acting drugs on serotonin and amino acid metabolism. However, caution must be exercised in extrapolating any results directly to the mammalian brain where, for example, the active principle may be a major metabolite of the drug and where the pharmacological action may be due to a specific effect on a discrete brain area.

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