

An altered metabolism of dopamine in the striatal tissue of mice made aggressive by isolation

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In the mouse brain, dopamine is metabolized to 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA). Roffler-Tarlov, Sharman & Tegerdine (1971) have proposed that DOPAC is formed within the dopaminergic neurone, that HVA is formed at a different site and that little DOPAC is metabolized to HVA.

a fresh cage for 15 minutes. The mice were then killed and the striatal tissues analysed for HVA and DOPAC (Murphy, Robinson & Sharman, 1969) and for dopamine (Lavery & Sharman, 1965). Table 1 shows that in the mice made aggressive by isolation there was a significant increase in the striatal concentrations of DOPAC but no change in the striatal concentration of HVA when they were exposed to a second mouse or when they were placed in a fresh cage. 'Aggregated' mice did not show this response but when 'aggregated' mice were exposed to a temperature of -10°C or restrained for 15 min there was an increase in the striatal concentration of HVA with little or no change in the concentration of DOPAC. The increase in the concentration of DOPAC in the 'isolated' mouse exposed to a second mouse has also been observed using a

Table 1 The concentrations of dopamine, 4-hydroxy-3-methoxyphenylacetic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum of the mouse

Group	Concentration ($\mu\text{g/g}$ tissue)		
	Dopamine (whole brain)	DOPAC (striatum)	HVA (striatum)
1. <i>'Aggregated' mice</i>	0.83 \pm 0.05 (17)	0.26 \pm 0.02 (14)	0.27 \pm 0.02 (14)
2. <i>'Aggregated' mice</i> transferred in groups of four to a fresh cage for 15 min		0.27 \pm 0.01 (10)	0.27 \pm 0.01 (10)
3. <i>'Isolated' mice</i>	0.75 \pm 0.05 (15)	0.27 \pm 0.02 (13)	0.26 \pm 0.01 (13)
4. <i>'Isolated' mice</i> transferred to a fresh cage for 15 min		0.34 \pm 0.01 (7) (4 vs 3*)	0.28 \pm 0.01 (8)
5. <i>'Isolated' mice</i> exposed to 'aggregated' mice for 15 min	0.76 \pm 0.05 (11)	0.38 \pm 0.02 (17) (5 vs 3**)	0.30 \pm 0.02 (17)
6. <i>'Isolated' mice</i> exposed to 'isolated' mice for 15 min		0.37 \pm 0.03 (6) (6 vs 3**)	0.28 \pm 0.02 (6)

The italicized descriptions indicate the mice to which the values in the table refer.

Comparisons of DOPAC concentrations: Student's *t* test **P* < 0.02, ***P* < 0.01.

Number of observations in parenthesis; each observation was made on the pooled tissues from four mice.

Male, albino mice were housed singly for 4 weeks in normal daylight and for at least 2 weeks under reversed lighting (red light 10.00-22.00 h). This treatment caused the mice to show intense aggressive behaviour when exposed to a second mouse. Corresponding groups of aggregated mice were kept under the same conditions.

Two mice were placed in a fresh cage and observed for 15 minutes. There were two 'isolated' mice, two 'aggregated' mice, or one from each source. Mice were also placed singly or grouped in

gas-liquid chromatographic method (Pearson & Sharman, unpublished results) for the estimation of HVA and DOPAC. Some implications of these observations will be discussed.

D.A.H. was supported by the Wellcome Trust and J.D.M.P. by the Medical Research Council.

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An analysis of the catecholamine content of the salivary gland of the cockroach

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There is evidence that dopamine might be a neurotransmitter in the salivary gland of the cockroach *Nauphoetia cinerea* Olivier. Electrical stimulation of the salivary nerve results in hyperpolarization of the gland cell membrane (House, 1973). This effect is mimicked by adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine (5-HT). The effect of the catecholamines or nerve stimulation, but not that of 5-HT, is antagonized by phentolamine. The gland, however, does not respond to some other α - or β -adrenoceptor stimulating drugs (House, Ginsborg & Silinsky, 1973). Microspectro-fluorimetry has indicated the presence of a catecholamine in the nerve terminals in the gland (Bland, House, Ginsborg & Laszlo, 1973). Using a radiochemical assay for the simultaneous measurement of adrenaline, noradrenaline and dopamine we have investigated the catecholamine content of the cockroach salivary gland. The method is a development of that described by Cuello, Hiley & Iversen (1973). Twelve salivary glands, dissected as described by House (1973), were homogenized in 20 μ l 0.1 M ice-cold perchloric acid, 10 μ l saturated KCl solution added and the mixture centrifuged. 10 μ l of the supernatant was incubated for 15 min at 37°C with 25 μ l of a mixture, slightly modified from that described by Cuello *et al.* (1973) containing [³H]-methyl S-adenosyl methionine and catechol-O-methyl transferase (prepared from pig liver). After the incubation, 25 μ l of a solution containing metanephrine, normetanephrine and 3-methoxytyramine (10 mg/ml) was added followed by 10 μ l 1 M perchloric acid and 20 μ l saturated KCl solution. The mixture was extracted three times with ethyl acetate (250 μ l) to reduce reagent

blank radioactivity. A sample (50 μ l) of the aqueous phase was treated twice with 10 μ l acetic anhydride and solid NaHCO₃ to acetylate the methoxy derivatives of the catecholamines; these were extracted into ethyl acetate and separated by paper chromatography (see Sharman, 1971). The appropriate regions of the chromatogram were visualized by spraying with conc. ammonia solution, then with Folins and Ciocalteu's solution and finally with conc. ammonia solution. The chromatogram was cut into consecutive 1 cm portions and each portion placed in 10 ml Unisolve scintillation fluid for measuring radioactivity.

Three radioactive regions, not present on reagent blank chromatograms, were observed on chromatograms derived from salivary glands. The first did not coincide with any of the three radioactive regions derived from the catecholamines. The second lay beneath the region corresponding with acetyl normetanephrine but the *R_f* of the peak of the radioactivity (0.299 ± 0.009 (s.e. mean) $n = 8$) did not coincide ($P < 0.01$) with that (0.260 ± 0.008 (s.e. mean) $n = 8$) derived from added noradrenaline. The third region (*R_f* 0.745 ± 0.007 (s.e. mean) $n = 8$) coincided with that derived from added dopamine (*R_f* 0.737 ± 0.004 (s.e. mean) $n = 8$).

The salivary gland of the cockroach contains 0.55 ± 0.07 ng dopamine/gland (mean \pm s.e.; $n = 8$) but the evidence does not establish the presence of noradrenaline.

J.P.F. is supported by an A.R.C. Research Studentship.

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