Formation of Catecholamines and Acid Metabolites by *Octopus* Brain

Dopamine (DM) was the first catecholamine found in molluscan ganglia[1,2] and was considered to be the adrenergic transmitter in invertebrates[3]. Subsequently small concentrations of noradrenaline (NA) were detected in *Spisula*[1], *Helix*[4,5] and in several cephalopods[1,5,6]. Recent work has shown that ganglia of *Mercenaria* form labelled DM after incubation with radioactive tyrosine[7] or DOPA[7,8]. NA has not been detected in the ganglia of this lamellibranch[2] and no significant accumulation of labelled NA could be measured in these studies. The ganglia of *Octopus vulgaris* contain relatively high NA concentrations[6] and the present investigation describes the synthesis of labelled NA, as well as that of DM and dihydroxyphenylacetic acid (DOPAC).

O. vulgaris of 0.3–0.7 kg, either sex, were killed by decapitation and the brain or circumoesophageal ganglia dissected free. The brain was bisected sagitally and the optic lobes sectioned horizontally to allow penetration of the incubation medium. The slices were kept in oxygenated filtered sea water on ice until incubation commenced. The isotopes L-tyrosine-3, 5-^{3}H (52 Ci/mmole) and DL-dihydroxyphenylalanine-2-^{14}C (3.1 mCi/mmole) were diluted with sterile 3.5% NaCl which in the latter case contained ascorbic acid (5 mg/ml). The tissue was incubated at 20–24° in 2 ml oxygenated filtered sea water containing the isotopes at final concentrations of 15 µCi ^{3}H-tyrosine/ml or 0.2 µCi ^{14}C-DOPA/ml. After incubation periods of 30, 60 or 180 min the tissue was rinsed in sea water and weighed. Approximately 80% of the radioactivity remained in the medium after 3 h incubation. Tissues were homogenized in 0.1 *N* HCl and deproteinised with perchloric acid. Acid metabolites were extracted with ethylacetate and separated by paper chromatography[5] in *n*-butanol: acetic acid: water, 4:1:1. Once the acidic compounds had been extracted nitrogen was blown on to the remaining aqueous extract to eliminate ethylacetate. The amines were then acetylated and separated by paper chromatography[6]. Neither the estimations of the concentrations or radioactivity of the amines and acid metabolites were corrected for recoveries during the extractions. Chromatograms were cut into 1 cm strips and the radioactivity determined by liquid scintillation spectrometry.

Experiments on live animals were performed by exposing the cephalic aorta through a small dorsal incision in the mantle muscle and venous sinous under urethane anaesthesia. A Hamilton syringe was used to inject 50 µl of isotope into the aorta. Animals were either killed 5 min later or were resuscitated, after suturing the opening in the mantle, and then killed after 30, 60 or 180 min.

Results. The distribution of radioactivity after separation of the amines extracted from *Octopus* ganglia incubated for 1 h with ^{3}H-tyrosine is shown in Figure 1. The isotope was mainly localised in 2 peaks which corresponded in position to the acetates of DM and NA run on parallel marker strips. The peak nearest the origin was produced by DOPA which was acetylated and partially extracted with the amines.

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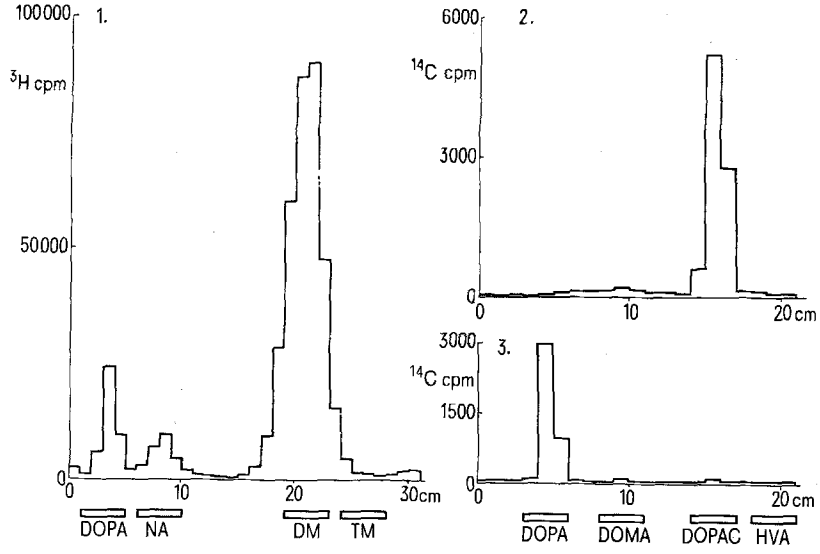

Fig. 1. Chromatography of total catecholamines from *Octopus* brain (0.47 g) incubated with ^{3}H-tyrosine for 1 h. Positions of standard dihydroxyphenylalanine (DOPA), noradrenaline (NA), dopamine (DM) and tyramine (TM) are marked below. The distance from the origin to the solvent front was 30 cm.

Fig. 2. Chromatography of total acid metabolites from *Octopus* brain (0.32 g) incubated with ^{14}C-DOPA for 1 h. Positions of standard dihydroxyphenylalanine (DOPA), dihydroxymandelic acid (DOMA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are marked below Figure 3.

Fig. 3. Chromatography of incubation medium after incubation of *Octopus* brain with ^{14}C-DOPA for 1 h. As in Figure 2 the distance from the origin to the solvent front was 20 cm.

Table I. Metabolism of ^{14}C-DOPA by *Octopus* brain in vitro

Time (min)	Radioactivity (dpm $\times 10^{-3}$/g brain)			Concentration (μg/g brain)		
	DM	DOPAC	NA	DM	DOPAC	NA
30	11.6 ± 1.1	11.9 ± 1.9	0.92 ± 0.1	8.78 ± 0.64	1.23 ± 0.12	1.95 ± 0.21
60	19.4 ± 2.8	15.3 ± 0.9	1.63 ± 0.2	8.31 ± 0.72	1.96 ± 0.21	1.97 ± 0.11
180	31.6 ± 3.9	30.4 ± 6.1	4.00 ± 0.4	7.52 ± 1.09	2.49 ± 0.60	1.95 ± 0.28

Values are means ± S.E.M. of 4 animals per group. Each brain was incubated with 0.2 μCi ^{14}C-DOPA/ml.

Chromatography of the acid metabolites (Figure 2) showed labelling of DOPAC but very little in the regions where homovanillic acid (HVA) and dihydroxymandelic acid (DOMA) standards ran. Separation of a sample of the medium after incubation (Figure 3) showed that only labelled DOPA was present in significant amounts.

The time-course of the in vitro formation of amines and acid metabolites by *Octopus* ganglia is shown in Table I. The amounts of radioactive DM, DOPA and NA increased steadily from 30 to 180 min although the synthesis of labelled DM and DOPAC was at all times greater than that of NA. The concentrations of the amines in the tissue did not change significantly during the course of the incubation.

After ^{14}C-DOPA had been injected in vivo the formation of radioactive DM, DOPAC and NA was examined at 5, 30, 60 and 180 min (Table II). Substantial labelling of NA and DM was obtained as early as 5 min after injection. The maximal accumulation of radioactive DM was detected at 30 min, lasted about 1 h and was reduced to nearly half its maximal level 3 h after the injection. The highest level of radioactive DOPAC was observed at 30 min coinciding with the labelling of DM. In contrast the level of radioactive NA showed no significant change from 5 to 180 min after precursor administration.

Discussion. The conversion of radioactive tyrosine and DOPA to the catecholamines DM and NA suggests that *Octopus* ganglia contain enzymes with tyrosine hydroxylase, DOPA decarboxylase and dopamine β-hydroxylase activity. Both in vivo and in vitro experiments show that DOPA is rapidly decarboxylated to DM which in turn is metabolized by a monoamine oxidase. Endogenous DOPAC has been detected in the optic lobes of *Octopus* but HVA has not been found[5], even after the administration of 200 mg/kg of l-DOPA (A. V. JUORIO, unpublished observation). The present results confirm that in *Octopus* ganglia radioactive DM is mainly metabolized to DOPAC and not to HVA, suggesting that DOPAC is normally removed from ganglia without methoxylation.

The results obtained in vitro are consistent with the synthesis of NA from DM, assuming that the lower rate of labelling of NA is due to the limiting rate of DM-β-hydroxylation. In vivo, however, the maximum period of DM labelling (30–60 min) is not followed by a corresponding increase in NA. The results suggest that there are 2 pools of DM with different specific activities. One pool of high specific activity in dopaminergic nerves, where DM has a transmitter function and is rapidly metabolized to DOPAC. The other, a low specific acticity pool, in noradrenergic nerves where DM functions as a precursor to NA. Assuming that most of the brain DM is in dopaminergic nerves the higher endogenous level of DM combined with the higher specific activity would imply that the dopaminergic nerves must also have a greater uptake of ^{14}C-DOPA than noradrenergic nerves. When a large exogenous pool of ^{14}C-DOPA is available, as is the case in the in vitro experiments, the synthesis of radioactive NA increases with time as does the synthesis of DM[9].

Table II. Metabolism of ^{14}C-DOPA by *Octopus* brain in vivo

Time (min)	Radioactivity (dpm $\times 10^{-3}$/g brain)		
	DM	DOPAC	NA
5	15.3 ± 2.7	0.93 ± 0.1	2.60 ± 0.4
30	25.5 ± 3.5	15.8 ± 1.4	1.90 ± 0.3
60	23.6 ± 8.2	5.17 ± 1.7	1.71 ± 0.4
180	14.1 ± 5.0	3.43 ± 0.9	1.77 ± 0.5

Values are means ± S.E.M. of 4 animals per group. Each animal was injected with 1 μCi ^{14}C-DOPA.

Resumen. Los ganglios cerebrales del pulpo (*Octopus vulgaris*) sintetizan la dopamina y la noradrenalina radioactivas luego de la administración in vivo o la incubación in vitro con sus precursores marcados. La dopamina es luego metabolizada a ácido dihidroxifenilacético mientras que el correspondiente derivado de la noradrenalina no fué encontrado.

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Fucosterol-24,28 Epoxide and 28-Oxo-β-Sitosterol as Possible Intermediates in the Conversion of β-Sitosterol into Cholesterol in the Locust *Locusta migratoria* L.

Phytophagous insects transform β-sitosterol (I) into cholesterol (VI)[1–4]. In the locust *Locusta migratoria* L., using 3-^{3}H precursors, the conversion of (I) has been shown to proceed through fucosterol (II) and desmosterol(V)[4,5]. This seems to indicate the simultaneous elimination of carbon atoms 28 and 29. Such a reaction should start with an oxidation, but it is rather difficult to predict at what carbon atom the attack would begin.