Distribution of type A and B MAO activities in CNS of rat and chick

Rat			Chick		
MAO activity (μmoles/g wet weight/30 min)			MAO Activity (μmoles/g wet weight/30 min)		
Regions	Type A (5-HT)	Type B (PEA)	Regions	Type A (5-HT)	Type B (PEA)
Spinal cord	3.18 ± 0.18	0.53 ± 0.16	Spinal cord	6.54 ± 0.47	2.41 ± 0.38
Cerebral hemisphere	4.82 ± 0.46	1.14 ± 0.25	Cerebral hemisphere	2.97 ± 0.47	7.78 ± 0.76
Cerebellum	4.24 ± 0.26	1.17 ± 0.15	Cerebellum	2.51 ± 0.54	3.09 ± 0.60
Medulla oblongata plus pons	4.18 ± 0.21	1.11 ± 0.11	Optic lobe	3.49 ± 0.55	4.52 ± 0.45
Brainstem	5.25 ± 0.51	1.36 ± 0.31	Brainstem	5.33 ± 0.44	10.05 ± 1.39

The concentration of the substrates, 5-HT and PEA, in the reaction mixture for the estimation of type A and B MAO activities was 0.2 m*M*. Means ± SD are given. Each value was obtained from 4 experiments.

surement of its fluorescence with excitation at 390 nm and emission at 490 nm. MAO activity towards PEA was expressed as μmoles of PEA metabolized in 30 min per g fresh tissue. The results are summarized in the Table.

In rat CNS, the reaction rate for PEA was smaller than that for 5-HT, and the pattern of distribution of MAO activity towards PEA was similar to that towards 5-HT. It should be noted that the activities are highest in the brainstem and lowest in the spinal cord. The pattern of distribution of MAO activity towards 5-HT observed in the present study is similar to that reported by HARADA et al.[12].

Differing from the results of rat brain, the activity towards PEA was generally higher than that towards 5-HT in chick brain. It is also noted that the ratio of type A to type B MAO activity is different in various areas of chick CNS. This may not be due to immaturity of CNS of newborn chick during its ontogenic development, but to the difference of animal species, since the metabolism of monoamines in CNS of newborn chick is thought to be well established and similar to that of the adult animal[15,16].

MAO activity towards 5-HT in chick spinal cord was higher than that in any region of chick brain, confirming our previous result[16], whereas the activity towards PEA in chick spinal cord was lower than that in any region of chick brain. Accordingly, a large ratio of type A to type B MAO activity was observed. This is also true in the case of rat spinal cord. These results suggest that MAO in the spinal cord may be associated with the sympathetic nervous system, since MAO in the sympathetic nerves was reported to be almost exclusively type A[17,18].

MAO activity towards PEA in chick cerebral hemisphere was markedly higher than that in any region of CNS with the exception of the brainstem. This should be noted while investigating the physiological significance of type B MAO as well as PEA in the brain.

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Occurrence of Octopamine in the Soft Tissues of the Gastropod Mollusc *Peristernia nassatula* Lamarck[1]

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Summary. The soft tissues of the gastropod mollusc *Peristernia nassatula* contain large amounts (30–60 μg/g) of octopamine, which, in all probability, is concentrated in the hypobranchial gland. Smaller amounts of the amine are present also in the soft tissues of the gastropods *Bulla adamsi* and *Latirus polygonatus*.

After its first identification in acetone extracts of the posterior salivary glands of *Octopus vulgaris*[2], octopamine (*p*-hydroxyphenylethanolamine) was detected in the nervous tissue and in other tissues of vertebrates and invertebrates[3–6]. Even human blood seems to contain detectable amounts of the amine[7]. According to SAAVEDRA et al.[8], octopamine may have neurotransmitter function of its own in the central nervous system of molluscs.

This communication describes the occurrence of large amounts of octopamine in extracts of the soft tissues of *Peristernia nassatula* Lamarck, a gastropod mollusc of the family Fasciolaridae.

Materials and methods. Three batches of *Peristernia nassatula* were collected near Dumaguete City (Negros Oriental, Philippines) in November 1973 (139 specimens, 39,1 g), January 1974 (53 specimens, 29,6 g) and July 1974 (197 specimens, 79 g). The whole soft tissues were removed from the living animals after cautious rupture of the shell and immediately extracted with 5 parts (w/v) of pure methanol. After 5–10 days the supernatant liquid was decanted and the tissue re-extracted with 5 parts of

80% methanol The extracts were mixed and filtered. Part of the crude extracts was studied as such, another part was submitted to chromatography on alkaline alumina column which was then eluted with descending concentrations of ethanol.

Both crude extracts and eluates were submitted to paper chromatography, thin-layer chromatography and high voltage electrophoresis. Synthetic octopamine was available for comparison.

Results and discussion. Following chromatography on alumina column, the phenol derivative supposed to be octopamine emerged in the 70% ethanol eluate. Its identification as octopamine is based on the following criteria: a) both the unknown phenol derivative and synthetic octopamine showed the same colour shades with the Pauly reagent (yellow), Gibbs reagent (sky blue) and diazotized *p*-nitroaniline (lillac). b) on high voltage electrophoresis, the unknwon substance showed the same mobility towards the cathode as synthetic octopamine: $E_{1.2}$ = 0.9 *p*-tyramine, $R_{5.8}$ = 0.89 *p*-tyramine. c) on paper chromatography, Rf values were exactly the same for the unknown substance and for synthetic octopamine in 4 solvent systems: 0.42–0.49 in *n*-butanol:acetic acid:water (4:1:5); 0.21–0.23 in *n*-butanol: 35% methylamine (8:3), 0.74–0.78 in 1-penthanol:pyridine:water (4:4:1), and 0.77–0.80 in 20% KCl. d) superimposable Rf values were obtained also in thin-layer chromatography on silica gel: 0.12–0.14 in benzene:ethanol:methylamine (22:7:1); 0.45–0.47 in *n*-butanol-acetic acid:water (4:1:5).

The contents of octopamine in the different batches were 30, 60 and 45 µg per g fresh tissue, respectively.

In about 6–7 specimens of *Peristernia*, the hypobranchial body was separated from the remaining soft tissues (0.4 g versus 2.0 g) and analyzed. It contained 5 times as much octopamine as the other tissues (125 versus 25 µg/g). Thus, it appears probable that, like other active compounds (murexine, dihydromurexine, purple prepigments) also octopamine is concentrated in the hypobranchial gland.

In addition to octopamine, the soft tissues of *Peristernia* contained small amounts of imidazolepropionic acid, its methyl ester, and choline. It is possible that all these compounds are postmortal artifacts deriving from dihydromurexine by hydrolysis and transmethylation processes[9].

Small amounts of octopamine could be detected also in the soft tissues of other gastropod molluscs of the Philippines, such as *Bulla adamsi* (5–7 µg and 2 µg per g total soft tissues, respectively, in 2 different batches) and *Latirus polygonatus* (4 µg per g total soft tissues).

It appears that octopamine originates in tissues from *p*-tyramine by the action of dopamine-β-hydroxylase[10]. *p*-Tyramine has not been found in *Peristernia nassatula*, but it is present in considerable amounts (30 µg/g) in the soft tissues of *Bulla adamsi*.

The function of octopamine in the hypobranchial body of gastropod molluscs is as obscure as that in the posterior salivary glands of octopoda. It would be of interest to check whether this potential adrenergic transmitter could be used here for defense or for capturing prey.

After 21 days of incubation, 50 µCi $Na_2{}^{35}SO_4$ (NEN, specific activity 859 mCi/m*M*) were added to the medium

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Synthesis of Sulfated Glycosaminoglycans by the Three Cell Types of the Rabbit Cornea in Culture[1]

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Summary. Rabbit corneal cells were cultivated for 21 days and then exposed to $Na_2{}^{35}SO_4$, a precursor of sulfated glycosaminoglycans (GAG). All 3 cell types of the cornea, the fibroblasts, the epithelial as well as the endothelial cells, synthesize GAG. The fractionation-patterns of the epithelial and endothelial GAG are almost identical and differ clearly from the one of fibrolastic GAG.

Biosynthesis of collagen and glycosaminoglycans (GAG) is thought to be the domain of mesodermal cells, but evidence exists that also ectodermal cells, i.e. the epithelium of the chick and the rabbit cornea, produce these matrix components[2–6]. It is not yet established, however, whether all three cell types of the rabbit cornea do synthesize GAG and how far the fractionation-patterns of the GAG synthesized by these cells differ from each other.

Material and methods. Epithelial and fibroblastic cultures from the rabbit cornea were made as described elsewhere[3]. Endothelial cultures were prepared by treating the Descemet's membrane, stripped off from the corneal stroma, with 0.005 *M* Na_2EDTA and 1.25% trypsin for 30 min at 37 °C. These substances were dissolved in minimal Eagle's medium, Hanks BSS. The nutrient medium was TC 199 containing 20% fetal calf serum and antibiotics.

The cells originated from the corneas of 5 rabbits. All cultures were primary, 21 days old and at about saturation density at the time of labelling. Thus the experimental conditions were identical for all three cell types.

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