lite of DHA, fr. 5, was in addition recrystallized to constant isotope ratio (table 2).

In all 30-min DHA incubations carried out either without cofactors or with NADP the main metabolite was androstenedione. The utilization of the substrate by $\Delta^5 3\beta$HSD was increased when NADP was added in both experimental groups. The recovery of androstenedione, however, was higher in the incubations performed during breeding, suggesting increased $\Delta^5 3\beta$HSD activity in the testicular tissue of these animals.

When the incubation time was doubled to 1 h and NADP/NADPH added in equimolar concentrations, testosterone was found as the main metabolite of DHA during breeding but not after breeding.

Comparable observations were made when androstenedione was used as the substrate. In the 1-h incubations with NADP/NADPH testosterone was produced in the same proportions as in DHA incubations and the production declined similarly after breeding.

These observations suggest that the activity of 17βHSD may be regulated for the needs of testicular androgen production leading to the increased testosterone formation during breeding.

Previous studies in other urodele Amphibia *(Pleurodeles waltlii, Triturus cristatus carnifex)* have also demonstrated that testosterone is the major androgen produced in vitro by the testis[2,3]. In peripheral plasma of *Ambystoma tigrinum, Pseudoeyrycea smithi*[12] *Necturus maculosus*[13] 5α-DHT can be found in relatively minor amounts compared to testosterone. In accordance with these findings 5α- or 5β-DHT were not found in our studies. On the other hand testicular tissue of anuran species has been found to form large amounts of 5α-DHT from exogenous precursors during breeding[1,4,6,7]. This ability differs therefore from that observed in urodele Amphibia and among all other orders of vertebrates[6,14].

In conclusion the formation of biologically active androgens from DHA and androstenedione in testicular homogenates of *T. vulgaris* showed a clearcut variation between breeding, aquatic newts and non-breeding, terrestrial newts. The difference was most distinct in relation to 17βHSD activity. Testosterone was quantitatively the most important metabolite of both substrates during breeding.

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The presence of β-phenylethylamine, *p*-tyramine, *m*-tyramine and tryptamine in ganglia and foot muscle of the garden snail *(Helix aspersa)*[1]

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Summary. The concentrations of β-phenylethylamine, *p*-tyramine, *m*-tyramine, *m*-octopamine and tryptamine in the ganglia or foot muscle of *Helix aspersa* range from < 0.6 to 11 ng/g. *p*-Octopamine levels are higher in ganglia (327 ng/g) than in foot muscle (4.1 ng/g). Dopamine and 5-hydroxytryptamine range from 840 to 2710 ng/g while their acid metabolites, 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid and 5-hydroxyindoleacetic acid range from < 20 to 130 ng/g.

It has been known for quite some time that the ganglia of gastropods contain relatively high concentrations of dopamine (DA) or 5-hydroxytryptamine (5-HT)[3-5], and this has been confirmed by a histochemical fluorescence technique[6]. In addition, snail ganglia contain relatively large concentrations of *p*-octopamine and/or *m*-octopamine[7].

The snail ganglia possess low monoamine oxidase (MAO) activity[8-11] and it has been suggested that the effects of DA or 5-HT in these snail ganglia are ended by their re-uptake into nerve terminals or by diffusion into the blood[12,13]. The low concentration of 5-hydroxyindoleacetic acid (5-HIAA) observed in ganglia of *Helix*[14] support these interpretations.

In this work, we report the identification of β-phenylethylamine (PE), *p*-tyramine (*p*-TA), *m*-tyramine (*m*-TA), *p*-octopamine (*p*-OA), *m*-octopamine (*m*-OA) and tryptamine (T) in garden snail *(Helix aspersa)* ganglia and foot muscle. For comparison, the concentrations of DA, 5-HT and those of their respective acid metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (HVA) and 5-HIAA) were also determined.

Materials and methods. Helix aspersa (body weight including shell 7–10 g) were supplied by College Biological Supplies, Bothel, Washington, USA. They were fed on fresh lettuce leaves and kept active for at least 7 days before an experiment. Experiments were conducted throughout the year and hibernating animals were induced to become active by keeping them at room temperature, in a moist atmosphere, until they emerged from their shells.

The determinations were carried out using the whole circumoesophageal ganglia (COG) including the cerebrosuboeso-

phageal connectives and surrounding capsula; individual ganglia weighed 10–13 mg. Samples of the foot muscle were obtained from the anterior medial portion and weighed about 40–60 mg.

The pooled ganglia from 12 snails or a central strip from the anterior part of the foot muscle from one snail were used for the determinations of PE, *p*-TA, *m*-TA or T. The amines in the tissue homogenates were derivatized with 5-dimethylamino-1-napthalenesulphonyl (dansyl) chloride and the resultant derivatives extracted into toluene:ethyl acetate (9:1, v/v), evaporated to a small volume and separated chromatographically in two different unidimensional systems. The first system, toluene:ethyl acetate (5:2, v/v) was the same for PE, *p*-TA, *m*-TA and T. The second system was toluene:triethylamine (5:1 v/v) for PE; toluene:triethylamine: methanol (50:5:1, v/v) for *p*- and *m*-TA; or toluene:triethylamine:methanol (50:5:7, v/v) for T. The estimations of PE, *p*-TA or T were carried out by a high resolution mass spectrometric selected ion monitoring (integrated ion current) technique using the corresponding deuterated amines (which were added to the homogenate at the beginning of the experiment) as internal standards. Complete details concerning this procedure have been described[15–18].

The determination of *p*-OA and *m*-OA were carried out using 2 pooled ganglia or anterior foot muscle samples. Tissue homogenates were prepared in 100 mM Tris buffer in the presence of 1.3 mM pargyline and then heated to denature the proteins. Portions of the supernatant corresponding to about 10 mg of tissue were then incubated with partially purified phenylethanolamine-N-methyltransferase (E.C. 2.1.1.) and tritiated S-adenosylmethionine which served as a methyl donor[19]. Internal standards were obtained by adding supplements of *p*- or *m*-octopamine to a portion of each tissue homogenate. The *p*-synephrine and *m*-synephrine formed in the enzymatic reaction were extracted with ethyl acetate and evaporated to dryness; they were dissolved in sodium carbonate solution and dansyl chloride added[20]. After extraction with toluene:ethyl acetate (9:1, v/v), the extracts were evaporated to a small volume and separated chromatographically in 2 different unidimensional systems. The first system was chloroform:n-butyl acetate (5:2, v/v) and the second toluene:triethylamine:methanol (50:5:1, v/v).

Table 1. The concentrations of some trace amines in the circumoesophageal ganglia (COG) and the foot muscle of *Helix aspersa*

	COG (ng/g)	Foot muscle (ng/g)
PE	0.6 ± 0.2 (6)	0.8 ± 0.5 (7)
p-TA	7.5 ± 0.8 (10)	5.7 ± 0.7 (5)
m-TA	1.1 ± 0.3 (10)	0.6 ± 0.2 (5)
p-OA	327 ± 40.8 (16)	4.1 ± 0.4 (7)
m-OA	1.5 ± 0.5 (14)	< 0.6 (7)
T	6.4 ± 0.9 (13)	11 ± 2.9 (10)

Values are means (± SEM, number of experiments in parentheses) in ng/g of fresh tissue.

Table 2. The concentrations of dopamine (DA) 5-hydroxytryptamine (5-HT) and some of their acid metabolites in the circumoesophageal ganglia (COG) and the foot muscle of *Helix aspersa*

	COG (ng/g)	Foot muscle (ng/g)
DA	2200 ± 90 (26)	840 ± 40 (11)
DOPAC	70 ± 10 (12)	130 ± 50 (5)
HVA	< 20 (12)	< 40 (5)
5-HT	2250 ± 110 (17)	2710 ± 260 (11)
5-HIAA	60 ± 20 (12)	30 ± 10 (5)

Values are means (± SEM, number of experiments in parentheses) in ng/g of fresh tissue.

DA and 5-HT were determined using a high performance liquid chromatographic system[21] with an Altex Ultrasphere C_{18} ion pairing column (particle size 10 µm and internal diameter 3.9 mm). The solvent system consisted of 0.1 N nitric acid titrated to pH 2.7 with sodium hydroxide, 20 µg/ml of disodium ethylenediamine tetra-acetate and 2% methanol. A flow rate of 1 ml/min was maintained throughout the analyses. For DOPAC, HVA and 5-HIAA the concentration of methanol was increased to 18–20% in order to improve the separation of the compounds. Calibration curves were prepared each day an analysis of the tissue extracts was made. The internal standards were N-methyldopamine for DA, isoproterenol for 5-HT and 3,4-dihydroxycinnamic acid for DOPAC, HVA and 5-HIAA. An electrode potential of 0.72 V with respect to the Ag/AgCl reference electrode was used. The injection of 1–5 µl of the extract and a sensitivity range of 10–20 nA/V gave a good signal for the measurement of peak heights.

Results. The concentrations of PE, *p*-TA, *m*-TA, *p*-OA, *m*-OA and T in the ganglia (COG) and foot muscle of *Helix aspersa* are given in table 1. In the ganglia the predominant trace amine was *p*-OA with values exceeding 300 ng/g, the next highest were *p*-TA and T at 6–8 ng/g while lowest values were observed for *m*-TA, *m*-OA and PE (0.6–1.5 ng/g) (table 1).

In the foot muscle, T exhibited the highest values (11 ng/g), followed by *p*-TA and *p*-OA (4–6 ng/g) and PE, *m*-TA and *m*OA (< 0.6–0.8 ng/g) (table 1). The concentrations of DA and 5-HT (table 2) in the ganglia (COG) (respectively 2200 and 2250 ng) or foot muscle (respectively 840 and 2710 ng/g) confirm results obtained in earlier studies[14]. The concentrations of the acid metabolites (DOPAC, HVA and 5-HIAA) were generally low both in the COG (< 20–70 ng/g) and the foot muscle (< 40–130 ng/g) (table 2).

Discussion. The present experiments show that PE, *p*-TA, *m*-TA, *m*-OA and T are present in ganglia of *Helix aspersa* (table 1). The levels of these amines (0.6–7.5 ng/g) are substantially lower than those of DA or 5-HT (table 2)[6] and similar to those observed in the mammalian brain[22]. In contrast, the concentration of *p*-OA in snail ganglia (327 ng/g) is substantially higher than that of its *m*-isomer (1.1 ng/g) thus confirming earlier estimates[7] which did not discriminate *p*-OA from *m*-OA[7].

The comparison of the concentrations of some trace amines (PE, *p*-TA, *m*-TA, *p*-OA, *m*-TA, T) with those of the classical monoamine transmitters (DA, 5-HT) in neural tissues of some invertebrates or in rat brain (table 3) indicates that the concentrations of DA are generally high (ranging from 600 to 5950 ng/g); 5-HT is also high (400–4180 ng/g) with the exception of the arm nerve of the starfish *(Pycnopodia)* where its level is generally low (< 30 ng/g) (table 3). The concentrations of *p*-TA, *p*-OA or T are high in some invertebrates, low in others and in the rat brain (table 3). The concentrations of *p*-TA are higher in te *Octopus* optic lobe and those of *p*-OA in the nerves of the three invertebrate species here compared. The

Table 3. The concentrations of some neural monoamines in *Helix* ganglia, *Octopus* optic lobes, *Pycnopodia* arm nerve and rat whole brain

	Helix COG (ng/g)	*Octopus* Optic lobes (ng/g)	*Pycnopodia* Arm nerve (ng/g)	Rat Whole brain (ng/g)
PE	0.6	3.0[25]	4.4[26]	1.8[15]
p-TA	7.5	80.0[25]	2.2[26]	2[17]
m-TA	1.1	0.6[25]	< 0.8[26]	0.3[18]
p-OA	327	540[25]	160[26]	0.9[27]
m-OA	1.5	–	< 2[26]	0.2[27]
DA	2200	4690[25]	5950[26]	600[28]
T	6.4	< 0.6[25]	1250[26]	0.5[16]
5-HT	2250	4180[25]	< 30[26]	400[29]

The superscript indicates the source of reference. When no number is given, the results are from the present paper. * *p*- and *m*-OA were determined together[24].

starfish arm nerve contains a relatively high concentration of T (1250 ng/g). The concentrations of PE or m-TA are low (< 5 ng/g) in the neural tissue of the species so far studied (table 3). The concentrations of the acid metabolites of DA or 5-HT in *Helix* ganglia are quite low (table 2); these findings are in very good agreement with low MAO activity[8-11] and with the low concentrations for DOPAC, HVA or 5-HIAA in the ganglia of *Helix* reported in an earlier investigation[14]. Claims that the ganglia of *Helix aspersa* contain large concentrations of DOPAC and HVA[23] were not supported by the present (table 2) or earlier experiments[14]. The most striking difference in the concentration of monoamines between the ganglia and foot muscle of *Helix* is the low content of p-OA in foot muscle (table 1); the concentration of the other amines or metabolites in the foot muscle are of about the same order (tables 1 and 2) as those in the ganglia.

These results support the suggestion that the trace amines may possess a neurotransmitter or neuromodulatory role in snail ganglia or foot muscle and further confirm that monoamine oxidase has a limited role in the inactivation of some of these amines in this species.

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Ionophoric activity of the antibiotic X.14547 A in the mitochondrial membrane

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Summary. Release of Ca^{++}, Mg^{++} and K^+ by the carboxylic ionophore X-14547 A was studied in the mitochondrial membrane. A comparison was made with A.23187 (Calcimycin) and X.537 A (Lasalocid A) under the same experimental conditions. It was shown that in this test system X.14547 A is primarily a K^+ carrier comparable with X.537 A.

Antibiotics belonging to the large group of carboxylic polyethers generally act as monovalent cation carriers in membranes. However, a few are able to complex more specifically divalent cations and transport them through membrane phases; these make up a separate subgroup[1]. The anbitiotic X.14547 A, recently isolated from a strain of *Streptomyces antibioticus* NRRL 8167[2], is included in this subgroup as it carries Ca^{++} through a chloroform phase[3]. In methanol, the constants of formation of 2:1 neutral complexes (AH, A^- M^+ with alkali cations M^+ and 2 A^-, M^{++} with alkaline earth cations M^{++}) have been measured[4]. AH stands for the protonated form, A^- for the anion of the carboxylic antibiotic. X.14547 A has an unusual structure with a *trans*-butadienyl group connecting 2 heterocyclic systems as shown in figure A. Examination of models shows that it is not possible to obtain a folded structure forming a complexing cavity with a head-to-tail hydrogen bonding system as with A.23187[5] and X.537 A[6] which belong to the same subgroup and have molecular weights in the same range. This may explain the propensity of X.14547 A to form 2:1 complexes with M^+ in methanol as opposed to the others which give 1:1 associations[4]. Interestingly, the only crystalline structure available with this ionophore is also of the dimeric type AH, A^-M^+ where M^+ is replaced by the ammonium group of the chiral amine[7].

In a 3-phase system; aqueous phase/chloroform/aqueous phase the transport of calcium by X.14547 A is highly dependent on the pH of the compartment where the cation is extracted, the ionic flux being noticeable only for a pH above 8. This is dif-