Can L-dopa be a precursor of m-octopamine in the cephalic ganglions of the locust *Locusta migratoria* L.?

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Summary. Unexpectedly, the administration of L-dopa, alone or after reserpine depletion of octopamine, results in an increase of octopamine in the cephalic ganglions of *Locusta migratoria* L. This increase is exclusively due to m-octopamine and is correlated to dopamine and m-tyramine enhancement. This observation agrees with the basic idea of different biosynthetic pathways for p- and m-octopamine and indicates that unlike p-octopamine, m-octopamine synthesis is related to catecholic amine pathways.

In the Insecta nervous system, the direct incorporation of ^{14}C-tyrosine into octopamine, as well as the presence of dopamine hydroxylase, indicates that p-octopamine is biosynthesized from p-tyramine resulting from the decarboxylation of p-tyrosine[3-6]. Moreover, this route appears to be quantitatively important since in *Schistocerca,* the incorporation of ^{14}C-tyrosine is 10-fold higher in p-octopamine than in dopamine[3]. Yet the meta-isomer of octopamine has not been detected in the cerebral ganglions of either *Schistocerca gregaria*[3] or *Locusta migratoria*[7]. In order to study the interrelations of the different monoaminergic neurotransmitters in vivo and the importance of the tyrosine-octopamine pathway, we have examined the effects on octopamine synthesis of a treatment with precursors of this amine either associated or not with reserpine depletion[8].

Materials and methods. 1-day-old 4th stage larvae of both sexes and 7-day-old male adults of the gregarious *Locusta migratoria* L. were used in these experiments. 15 µg and 45 µg of reserpine (Serpasil, Ciba-Geigy Lab.) and/or 5 µg and 15 µg of L-dopa (ICN Pharmaceutical, free of precursor contaminants as checked according to different chromatographic procedures[9-11]) were given every 2 days to the larvae and the adults respectively. 2% acetic acid or 5% HCl N (solvents of reserpine and L-dopa respectively) were given to the controls. The larvae were treated during 4 or 6 days and the adults during 4 days. 2 h after the last injection, the animals were decapitated and the brain plus the optic and suboesophageal ganglions were rapidly dissected, frozen in liquid nitrogen and kept at −40 °C until used.

Octopamine with no separation of the m- and p-isomers was determined by the method of Molinoff et al.[12]. The dansylation of the methylated amines allowed the identification and separation of m- and p-octopamine[10-12]. Dopamine and noradrenaline were determined as described by Dymond and Evans[15]. m-tyramine was assayed according to Tallman et al.[16]; the extracts were prepared as for octopamine assays and the hydroxylated and N-methylated products were isolated as described for octopamine[10-12].

Results and discussion. As shown in table 1, reserpine treatment results in a large depletion of octopamine in the *L.migratoria* cephalic ganglions, whereas unexpectedly, treatment with L-dopa, either alone or after reserpine depletion, produces a slight increase or a partial replenishment of the octopamine stores. Moreover the octopamine content increases with age in the cephalic ganglions of animals in the 4th larval stage. This observation is in good agreement with experiments already reported concerning the whole locust head[13].

Although only p-octopamine has been reported in the cerebral ganglions of the locust[3,6], investigations have been performed towards the identification of m-octopamine in the cerebral ganglions of these treated locusts. As shown in table 2, no m-octopamine can be detected in the cephalic ganglions of controls or reserpine-treated locusts, and there is a significant difference in the amount of p-octopamine found in males and females at the end of the 4th larval stage. However, the administration of L-dopa, alone or consecutively to reserpine pretreatment, results in the exclusive increase of the sole m-octopamine. In the hemo-

Table 1. Octopamine content in the cephalic ganglions of *Locusta migratoria* treated at the 4th larval stage and imaginal stage either with reserpine, L-dopa or reserpine + L-dopa

Stage	Sex	Controls	Reserpine	L-Dopa	Reserpine + L-dopa
5-6-day-old 4th larvae	♀	5.1 ± 0.7[a]	1.1 ± 0.25	7.9 ± 0.3	2.7 ± 0.53[a]
	♂	4.8 ± 0.9[a]	0.5 ± 0.07	5.2 ± 0.42	1.07 ± 0.09
7-8-day-old 4th larvae	♀	20.3 ± 4[b]	0.63 ± 0.05	21.8 ± 2.1	7.25 ± 0.82
	♂	13.2 ± 2[c]	0.53 ± 0.11	13.1 ± 0.7	4.5 ± 0.50
Adults	♂	11.1 ± 1.7[d]	0.33 ± 0.7[a]	15.7 ± 0.5	3.7 ± 0.59

The octopamine values are expressed in pmole per piece of tissue ± SEM obtained from assays on 4 pools (except [a] 8, [b] 10, [c] 13 and [d] 16) each containing 2 pieces of tissue.

Table 2. p- and m-Octopamine content in the cephalic ganglions of *Locusta migratoria* treated at the 4th larval stage and imaginal stage either with reserpine, L-dopa or reserpine + L-dopa

Stages	Sex	Controls		Reserpine		L-Dopa		Reserpine + L-dopa	
		p-OA	m-OA	p-OA	m-OA	p-OA	m-OA	p-OA	m-OA
5-day-old 4th larvae	♀	4.5 ± 0.7[b]	n.d.	1.2 ± 0.2	n.d.	4.1 ± 0.9	2.7 ± 0.5	1.1 ± 0.15	2.0 ± 0.18
	♂	4.5 ± 0.9[b]	n.d.	0.45 ± 0.05	n.d.	3.7 ± 0.7	1.0 ± 0.2	0.61 ± 0.06	0.6 ± 0.05
7-day-old 4th larvae	♀	18.1 ± 3.5[a]	n.d.	0.51 ± 0.07	n.d.	17.3 ± 1.3	3.7 ± 0.6	0.57 ± 0.05	3.1 ± 0.7[b]
	♂	12.2 ± 2[c]	n.d.	0.45 ± 0.09	n.d.	12.0 ± 0.9	1.1 ± 0.05	0.50 ± 0.04	1.7 ± 0.3[b]
Adults	♂	11.5 ± 1.5	n.d.	0.31 ± 0.05	n.d.	11.1 ± 0.95	4.4 ± 0.6	0.35 ± 0.07[b]	4.7 ± 0.4[b]

The m- and p-octopamine (m-OA, p-OA) values are expressed in pmole per piece of tissue ± SEM obtained from assays on 4 pools (except [a] 6, [b] 8 and [c] 10) each containing 4 pieces of tissue. n.d., not detectable.

Table 3. Dopamine and noradrenaline in the cephalic ganglions of *Locusta migratoria* treated at the 4th larval stage and imaginal stage either with reserpine, L-dopa or reserpine + L-dopa

Stages	Sex	Controls		Reserpine		L-Dopa		Reserpine + L-dopa	
		DA	NA	DA	NA	DA	NA	DA	NA
5-day-old 4th larvae	♀	3.5 ± 0.4	n.d.	0.31 ± 0.02	n.d.	15.5 ± 1.3	0.5 ± 0.04	7.5 ± 0.8	n.d.
	♂	3.7 ± 0.4	n.d.	0.30 ± 0.01	n.d.	15.1 ± 1.1	0.55 ± 0.04	6.9 ± 0.7	n.d.
7-day-old 4th larvae	♀	6.1 ± 0.6	1.05 ± 0.08	n.d.	n.d.	28.3 ± 3	1.55 ± 0.15	8.9 ± 1.1	0.85 ± 0.07
	♂	5.5 ± 0.4	1.0 ± 0.1	n.d.	n.d.	26.1 ± 1.5	1.65 ± 0.10	9.1 ± 0.9	0.90 ± 0.08
Adults	♂	4.95 ± 0.41	1.4 ± 0.1	n.d.	n.d.	29.5 ± 3	2.05 ± 0.31	9.9 ± 1.0	0.69 ± 0.08

Dopamine (DA) and noradrenaline (NA) determinations are expressed in pmole per piece of tissue ± SEM obtained from assays on 4 pools each containing 2 pieces of tissue. n.d., not detectable.

lymph of control animals p-octopamine content is less than 10 ng/g and m-tyramine and m-octopamine are not detectable. These haemolymphatic octopamine contents are not changed after L- dopa or L-dopa plus reserpine administration.

These observations suggest different hypotheses. They may reflect a very particular biosynthesis pathway for m-octopamine in the nervous system of the locust and this pathway may involve L-dopa, dopamine and m-tyramine. Such a route has already been mentioned in mammals by Boulton[14]. On the other hand, the observations may represent a control of dopaminergic axons over octopaminergic synthesis, since the treatment is chronic. This second hypothesis is still under investigation and cannot be answered at present[8].

In order to check the first hypothesis, dopamine and noradrenaline were determined in the same treated locusts (table 3). The difference due to sex between the amounts of dopamine and noradrenaline respectively are not significant. However, as already described for octopamine, there is an increase in the concentration of both amines at the end of the 4th larval stage. As expected, the sole administration of L-dopa results in an important increase (about 5-fold) in the concentration of dopamine in all treated animals; concerning noradrenaline, the increase is very small after the same treatments, though significant ($p < 0.001$) in all cases. The administration of L-dopa plus reserpine is followed by a replenishment of dopamine; the very low levels in noradrenaline are only partially recovered if the animals are pretreated with reserpine before L-dopa administration.

Such observations lead to the conclusion that m-octopamine biosynthesis may involve noradrenaline or more probably dopamine as precursors. Since the immediate precursor of m-octopamine is supposed to be m-tyramine, it was of interest to search for a possible increase in the amount of m-tyramine consecutively to the administration of L-dopa alone or in combination with reserpine pretreatment.

As shown in table 4, no m-tyramine is detected either in the control ganglions or in the ganglions of reserpine-treated locusts. However, significant amounts of m-tyramine are found after treatment with L-dopa, and some of them are also present in the late 4th larvae and in the adults after the treatment with reserpine plus L-dopa. As mentioned above no change is observed in the cephalic p-octopamine content after treatments with L-dopa or reserpine plus L-dopa.

Table 4. m-Tyramine content in the cephalic ganglions of *Locusta migratoria* treated at the 4th larval stage and imaginal stage either with reserpine, L-dopa or reserpine + L-dopa

Stages	Sex	Controls	Reserpine	L-Dopa	Reserpine + L-dopa
5-day-old 4th larvae	♀	n.d.	n.d.	2.1 ± 0.17	1.7 ± 0.10[a]
	♂	n.d.	n.d.	2.0 ± 0.10	1.5 ± 0.10[a]
7-day-old 4th larvae	♀	n.d.	n.d.	5.7 ± 0.6	4.3 ± 0.37[a]
	♂	n.d.	n.d.	7.1 ± 0.65	4.7 ± 0.3[a]
Adults	♂	n.d.	n.d.	5.9 ± 0.6	3.9 ± 0.3[a]

m-Tyramine contents are expressed in pmole per piece of tissue ± SEM obtained from assays on 4 pools each containing 5 pieces of tissue (except [a] 8). n.d., not detectable.

From these observations, it clearly appears that in the cerebral ganglions of *L. migratoria*, m-octopamine can be produced under the effect of L-dopa administration. The subsequent increase in the concentrations of dopamine and m-tyramine agrees with the existence of a metabolic interconnexion from catecholic to phenolic amines. This observation is of great interest since it confirms the metabolic pathway of m-tyramine in mammals described by Boulton[14]. However, this route necessarily implies a dehydroxylating step from a catecholamine to a non-catecholamine[17]. Moreover, the very small increase in the concentration of noradrenaline after L-dopa administration may indicate a larger conversion of dopamine to m-octopamine through m-tyramine (as published by Robertson and Juorio[3]) than from noradrenaline to m-octopamine.

L-Dopa has been shown to induce an octopamine release in the heart[17,18] and the brain[18] of the rat. The conversion of dopamine to m-tyramine and m-octopamine observed in our study could be an alternative explanation for those results.

Nevertheless, the significance of a normally apparently unused metabolic pathway to m-octopamine in the locust nervous system is definitely a matter of controversy.

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