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SEROTONERGIC AND DOPAMINERGIC INFLUENCE OF THE DURATION OF EMBRYOGENESIS AND INTRACAPSULAR LOCOMOTION OF *LYMNAEA STAGNALIS* L.*

ADRIENN FILLA, L. HIRIPI** and K. ELEKES

Department of Experimental Zoology, Balaton Limnological Research Institute, Hungarian Academy of Sciences, P. O. Box 35, H-8237 Tihany, Hungary

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The role of the dopaminergic and serotonergic system was studied during the embryonic development of the pond snail *Lymnaea stagnalis,* with special attention to the effect of dopamine and serotonin as well as their agonists and antagonists on the rotation of the veliger larvae, and to the effect of precursors and inhibitors of the synthetizing enzymes on the duration of the embryonic life. Serotonin, D-lysergic acid diethylamide and N,N-dimethyltryptamine increased at a concentration of 1 μM the rotation by 50%, 90% and 87% respectively, and among them D-Lysergic acid diethylamide was found to be the most potent agonist. Other serotonergic agonists and antagonists enhanced the frequency of the rotation (from 165% to 355%) at higher threshold concentrations in the following rank order: methysergid > tryptamine > 2,5-dimethoxy-4-iodoamphetamine > 5-carboxyamidotryptamine > bromo-lysergic acid diethylamide > 7-methyltryptamine. Application of 1-(2-methoxyphenyl) piperazine decreased the rotation by 76%. The reuptake inhibitor desipramine completely blocked the rotation and killed the embryos. Dopaminergic agonists accelerated the rotation by 62% to 233%, and their effect was ranged as follows: dopamine > apomorphine > m-tyramine ≅ p-tyramine. Chlorpromazine at 100 μM concentration killed the embryos. At a concentration of 100 μg/ml, tyrosine, the precursor of DA, slowed down the embryonic development by increasing the duration of the embryonic life from 8 to 10 days. Decarboxylase inhibitors, α-methyl-3,4-dihydroxyphenyl-alanine (25 μg/ml) and m-hydroxybenzylhydrazin (5 μg/ml), killed 50% of the embryos, meanwhile the rest hatched ten days later, compared to the control animals. The development was partially blocked by the serotonin precusor L-tryptophane (50 μg/ml). Trytophan hydroxylase blocker, p-chlorphenylalanine (50 μg/ml) resulted in a distortion of the body pattern of the embryos, and prevented the hatching of most (95%) of the animals.

Keywords: Serotonin – dopamine – embryogenesis – locomotion – *Lymnaea*

INTRODUCTION

The nervous system of Gastropods contains significant amounts of monoamines, the role of which has been demonstrated in the regulation of different physiological processes. In the *Lymnaea* CNS, the presence of serotonin (5-HT) and dopamine

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** Corresponding author; e-mail: hiripi@tres.blki.hu

(DA) was detected by applying different methods, including fluorimetric, HPLC, radioenzymatic and immunocytochemical [7, 13, 15, 22, 24]. In this pulmonate gastropod, 5-HT and DA were shown to modulate a variety of behavioral and physiological processes such as feeding [17, 18], respiration [28], learning [16] and growth [5]. The presence of monoamines in the developing embryos of different gastropod species, such as *Helisoma* [6, 11, 12], *Aplysia* [20, 21] *Haliotis* [1], *Crassostrea* [2, 3], *Phestilla* [26] and *Lymnaea* [4, 8, 19, 27, 31] has also been demonstrated.

In *Lymnaea* embryos, biochemically both amines can first be detected at the same embryonic stage (ca E15%). The later appearing, immuno- and histocytochemically visualized, cells containing DA were located in both the CNS and the periphery. The DA content increased gradually from E15% to E45% stages, whereas between stages E45%–E80% its concentration remained constant. In the last day of embryonic development, a rapid increase in DA content occurred [30]. The first 5-HT-ergic cells visualized immunocytochemically became apperent at approximately the E35% stage [19]. After this time, additional cells exhibited immunoreactivity, especially in the pedal and cerebral ganglia. The role of 5-HTergic system with particular attention to C1 cell, during embryogenesis has been studied in *Helisoma* [6, 12]. In contrast, there is no data on the role of the aminergic systems during the embryogenesis of *Lymnaea*. Therefore the aim of the present study was to investigate in details the role of 5-HT and DA during the embryonic development of *Lymnaea,* analysing the influence of 5-HTergic and DAergic agents on the rotations of the embryos and the length of intracapsular life.

MATERIALS AND METHODS

Egg masses were collected from laboratory populations of *Lymnaea stagnalis.* The embryos developed in 8–9 days within the capsule at 25 °C. Stages were expressed as a percentage of total embryonic development, according to Marois and Croll [19], wherein embryonic stage E0% corresponds to the first egg cleavage and E100% to hatching.

At stage E25–30% (late trochophore and early veliger stage) the locomotor behavior of the embryos can be characterized by continuos rotation within the egg capsule. Later, during metamorphosis (E50–70%), the animals gradually switch from rotational behavior to gliding by their foot on the inner surface of the egg capsule. In order to achieve a more direct effect of the chemicals applied, the egg capsules without egg mass were treated. When the effect of the agents on the rotation was investigated, single veliger (E30%) larvae was placed into a small chamber containing lake-water. After 5 min of incubation at room temperature, the rotation was counted for an additional 2 min. Thereafter the lake-water was changed for a solution containing the drug, and following 5 min of incubation the rotation was counted for an additional 2 min. Experiments were carried out in triplicates, i.e. each treatment was performed in three parallel samples.

When investigating the effects of precursors and enzyme inhibitors on the duration of the embryonic life, the egg mass was divided into two parts. One part containing control embryos was placed into lake-water, whereas the other part of the embryos was placed into different solutions containing drugs. Development of the embryos was continuously monitored under stereo microscope. Drugs applied were dissolved in filtered lake-water, and each solution was freshly prepared immediately before application. The drugs used in this study were obtained from commercial source and were as follows: apomorphine, bromo-lysergic acid diethylamide (BOL), 5-carboxyamidotryptamine (5CT), p-chlorphenylalanin (pCPA), chlorpromazine, desipramine, 2,5-dimethoxy-4-iodoamphetamine (DOI), N,N-dimethyltryptamine (dimet-TP), dopamine (DA), 5-hydroxytryptamine (5-HT), m-hydroxybenzylhydrazin (mHBH), D-lysergic acid diethylamide (LSD), α-methyl-3,4-dihydroxyphenyl-alanine (α-mDOPA), 7-methyltryptamine (7-met-TP), methysergid, 1-(2-methoxyphenyl)piperazine (2-MPP), tryptamine (TP), L-tryptophane, tyramine, m-tyramine, tyrosine.

RESULTS AND DISCUSSION

The effect of the different aminergic pharmacons on the rotation of embryos is summarized in Table 1. The most effective agents were found to be LSD, 5-HT, and dimet-TP. These pharmacons accelerated the rotation at low (1 μM) threshold concentration. LSD increased the rotation by 40% compared to the effect of the 5-HT itself, whereas dimet-TP had the same effect as that of 5-HT. Other 5-HTergic substances also increased the frequency of the rotation at different threshold concentrations in the following rank order: methysergid > TP > DOI> 5-CT > BOL > 7met-TP. It is to be noted, that bromo-LSD had a significantly weaker effect than LSD. Using selective agonists and antagonists of 5-HT receptor subtypes, the identification of 5-HT receptor subclasses was not possible in gastropod neurons [29]. In the present experiments, it was found, that 5CT, the selective agonist of 5-HT_1, and DOI, the selective agonist of 5-HT_2 receptor, had only a weak stimulatory effect, whereas 2MPP, the another selective agonist of 5-HT_1 receptor inhibited by 76% the rotation of the embryos. At the same time, the application of the non-selective agonists, LSD and dimet-TP, resulted in a stronger effect on the rotation than the selective substances. Gerschenfeld and Paupardin-Tritsch [10] demonstrated that the TP analogues (TP, dimet-TP, 7-met-TP) significantly increased the activity of nerve cells in *Helix*. In the *Lymnaea* embryos, only dimet-TP proved to be highly active, whereas the activity of TP and 7-met-TP was 100 and 1000 times weaker, respectively. According to the findings of Goldberg et al. [12], 5CT and methysergide had the same effect as 5-HT on the rotation of *Helisoma* embryos. In contrast, we have found in *Lymnaea* embryos, that both agents affected less the rotation than 5-HT itself. Methysergide generally antogonized the 5-HT effect, meanwhile stimulating the rotation of both *Lymnaea* and *Helisoma* embryos [12].

Table 1
The threshold concentration of the pharmacons applied and their stimulatory effect on the rotation in percent of control

Pharmacons	Threshold concentration (μM)	Rotation in % of control mean ±S.E.M.
5-HT	1	150.00 ± 4.68
LSD	1	190.50 ± 3.51
N,N-dimethyltryptamine	1	186.95 ± 6.79
Methysergide	10	265.52 ± 5.97
Tryptamine	100	206.25 ± 9.37
DOI	100	175.00 ± 13.92
5CT	1000	455.55 ± 22.22
BOL	1000	280.39 ± 28.26
7-methyl-tryptamine	1000	186.95 ± 6.79
Dopamine	100	333.33 ± 38.19
Apomorphine	100	162.96 ± 13.98
p-Tyramine	1000	218.18 ± 23.62
m-Tyramine	1000	162.07 ± 2.98

In control experiments, the rotation was found to be 3.21 ± 0.68 (S.E.M.) rotation/min.

Dopaminergic substances enhanced the rotation rate of *Lymnaea* embryos by approx. 160–220%, however these drugs proved to be less effective than the 5-HTergic ones. The threshold concentrations were found to be at 100 μM for DA and apomorphine, and 1 mM for m-tyramine and p-tyramine. The threshold concentration of the DAergic antagonist chlorpromazine (100 μM) was found to be high and killed the embryos. The monoamine reuptake inhibitor desipramine blocked completely the rotation and killed the embryos at 50 μM threshold concentration, suggesting that the reuptake mechanism for monoamines, substituting the functional role of monoamino oxidaze enzyme in snails [14, 25], is present from the early development of the animals.

The effect of the amine precursors and enzyme inhibitors applied is shown in Figure 1. DA precursor tyrosine prolonged the intracapsular embryonic life by two days at a concentration of 100 μg/ml. The duration of development was also slowed down by the 5-HT precusor L-tryptophane (50 μg/ml); snails hatched two days later compared to the control (8 days). Decarboxylase blockers α-m-DOPA (25 μg/ml) and 3-HBH (5 μg/ml) killed almost 50% of the embryos, meanwhile the rest hatched ten days later compared to the control animals. Trytophane hydroxylase blocker, p-CPA (50 μg/ml) resulted in a distortion of the body pattern of embryos and reduced dramatically (90%) the number of surviving embryos. The p-CPA treatment of the *Helisoma* embryos also resulted in aberrant neuronal morphology during embryogenesis [11].

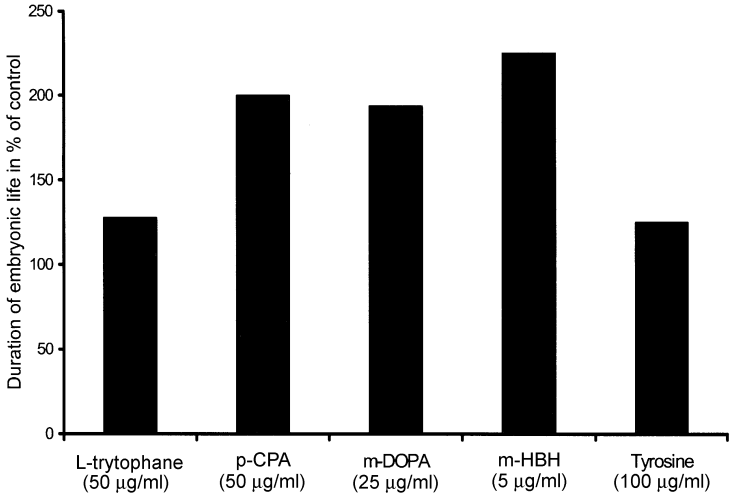

Fig. 1. Effects of 5HT and DA precursors and inhibitors of synthetizing enzymes on the duration of the embryonic life of *Lymnaea*

Our results clearly demonstrate that the 5-HTergic system plays a specific regulatory role in the early locomotor activity of the embryos. The non-selective 5-HT receptor agonists stimulated the rotation more significantly than the selective ones, suggesting that the vertebrate subtypes of the 5-HT receptor are not present in either the embryo or the adult animal (Hiripi et al., in preparation). The selective 5-HT synthesis inhibition resulting in aberrant embryonic morphology seems to be in a good correlation with previous immunocytochemical findings, showing the earlier (E30%) development of the 5-HTergic system than that of the DAergic elements, and the early innervation of the foot region by 5-HT-IR processes [30, 31]. The less effective influence of the DAergic agents on the rotation of the *Lymnaea* embryos refers to a different role of DA but locomotion in this early period of embryogenesis. Indeed, immuno- and histochemical observations revealed only DA-containing sensory cells but no efferent innervation at the periphery until finishing metamorphosis (E65% development) [30].

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